Development 126, 733-742 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
DEV5258

The trithorax group gene *osa* encodes an ARID-domain protein that genetically interacts with the Brahma chromatin-remodeling factor to regulate transcription

Martha Vázquez[1,2,*], Lisa Moore[3] and James A. Kennison[1]

[1]Laboratory of Molecular Genetics, National Institute of Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA
[2]Departamento de Genética y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, 62250, México
[3]Department of Biology, University of California, Santa Cruz, Santa Cruz, CA 95064, USA
*Author for correspondence at address 2 (e-mail: mvazquez@ibt.unam.mx)

Accepted 26 November 1998; published on WWW 20 January 1999

SUMMARY

The trithorax group gene *brahma* (*brm*) encodes the ATPase subunit of a chromatin-remodeling complex involved in homeotic gene regulation. We report here that *brm* interacts with another trithorax group gene, *osa*, to regulate the expression of the *Antennapedia* P2 promoter. Regulation of *Antennapedia* by BRM and OSA proteins requires sequences 5′ to the P2 promoter. Loss of maternal *osa* function causes severe segmentation defects, indicating that the function of *osa* is not limited to homeotic gene regulation. The OSA protein contains an ARID domain, a DNA-binding domain also present in the yeast SWI1 and *Drosophila* DRI proteins. We propose that the OSA protein may target the BRM complex to *Antennapedia* and other regulated genes.

Key words: Trithorax group (trx-G), *brahma*, *osa*, SWI/SNF complex, Chromatin-remodeling, Homeotic gene regulation, *eyelid* (*eld*)

INTRODUCTION

Homeotic genes specify the identities of segments during development. Many of the homeotic genes in *Drosophila* are found in two clusters, the Antennapedia complex (ANTC) and the bithorax complex (BXC) (Duncan, 1987; Kaufman et al., 1990), and are also referred to collectively as the HOM genes. The homeotic genes were first identified because mutations in them cause cells to form structures characteristic of another part of the body. For example, mutations in the HOM gene *Antennapedia* (*Antp*) cause the antennal cells to differentiate leg structures. The proteins encoded by the HOM genes share a 60 amino acid DNA-binding motif, the homeodomain, and function as positive or negative transcription factors of target genes.

Transcriptional regulation of the HOM genes is complex. The HOM genes have large *cis*-regulatory regions with redundant *cis*-regulatory elements (reviewed by Kennison, 1993). The establishment of individual HOM gene expression patterns early in embryogenesis is primarily controlled by the segmentation genes (for reviews see Ingham and Martínez Arias, 1992; Simon, 1995). However, many of the segmentation proteins disappear later in embryogenesis and other sets of genes are required to maintain the expression patterns of the HOM genes. Two groups of regulatory genes are required for maintenance of HOM gene expression. One group of genes, the Polycomb group (PcG), maintain repression of HOM genes. The second group of genes, the trithorax group (trxG), are positive regulatory factors required to maintain HOM gene expression (reviewed by Kennison, 1995; Simon, 1995). Many members of the trxG of genes were identified as suppressors of phenotypes caused by derepression of HOM genes (Kennison and Tamkun, 1988; Kennison and Tamkun, 1992). Because there are many expected regulatory steps involved in maintaining HOM gene function (such as transcriptional activation, posttranslational modification of HOM proteins, and expression of required HOM protein cofactors) the trxG genes are expected to be far more heterogeneous in function than the PcG genes, which all appear to repress transcription.

Kennison and Tamkun (1988) identified a dozen new trxG genes among which *brahma* (*brm*) is the most understood. BRM is the *Drosophila* homologue of the yeast SWI2/SNF2 protein and the human BRG1 and HBRM proteins**.** The BRM and SWI2/SNF2 proteins are most highly related within four segments: a DNA-dependent ATPase domain, a bromodomain, and two domains of unknown function. The DNA-dependent ATPase domain and one of the two domains of unknown function have been shown to be essential for BRM function, but the bromodomain appears to be dispensable (Elfring et al., 1998).

SWI2/SNF2 and other SWI/SNF genes were originally identified in yeast as a set of positive regulators of the *HO* gene (mating type switch, SWI) and the *SUC2* gene (sucrose non-fermenting, SNF) among other genes (reviewed by Winston and Carlson, 1992). SWI2/SNF2 can be biochemically isolated as an 11-subunit complex of approximately 2 megadaltons. Members of this SWI/SNF complex include SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, SNF6 (Cairns et al., 1994; Peterson et al., 1994), SWP73 (Cairns et al., 1996a), SNF11 (Treich et al., 1995), and TFG3/TAF30ANC1 (Cairns et al., 1996b). The SWI/SNF complex has DNA-dependent ATPase activity and several DNA-binding transcriptional activators have been identified whose action requires or is enhanced by the SWI/SNF complex (Gal4, Bicoid, the glucocorticoid receptor, the retinoic acid receptor, Sp1, USF, NF-κB, and others (Cairns et al., 1996a; Côtè et al., 1994; Laurent and Carlson, 1992; Ostlund-Farrants et al., 1997; Utley et al., 1997; Yoshinaga et al., 1992 and references therein). Nevertheless, many inducible genes do not require SWI/SNF function, even inducible genes that have been shown to undergo chromatin remodeling upon induction (Gaudreau et al., 1997). Why only a few genes require SWI/SNF function is not understood, however, it is possible that another protein (or proteins) target the complex to particular inducible genes.

In *Drosophila*, BRM is also found in a 2 MDa complex that contains at least seven subunits. At least four of these subunits are related to subunits of yeast chromatin remodeling complexes, including SWI/SNF and RSC (Dingwall et al., 1995; Papoulas et al., 1998). Previous genetic studies have suggested that trxG proteins might act in concert to regulate homeotic genes. However, the majority of the subunits of the BRM complex are not encoded by trxG genes, and the functional relationships among this group of homeotic gene regulators remains unclear.

We have used a genetic approach to try to identify other genes that functionally interact with the BRM complex in *Drosophila*. In the course of these studies, we discovered an unusually strong and specific genetic interaction between the trxG genes *brm* and *osa*. The *osa* (*osa*) gene was first identified as a trxG gene in the same genetic screens that identified *brm* (Kennison and Tamkun, 1988). In a search for mutations that interact genetically with *brm* mutations, we have isolated *osa* mutations. Here we show that *brm* and *osa* interact genetically and that they are both required for the function of specific *cis*-regulatory elements in the *Antp* gene. *osa* is not required for expression of all *brm*-regulated genes and this suggests that it is not essential for the function of the BRM complex itself, but may regulate its activity. How it may regulate function of the BRM complex is suggested by its protein sequence. The putative OSA protein sequence was recently published under the name EYELID (ELD) (Treisman et al., 1997) and contains a DNA-binding motif that may target the BRM complex to some (but probably not all) of its regulatory targets.

MATERIALS AND METHODS

Fly strains

Fly cultures and crosses were performed according to standard procedures. Flies were raised on a cornmeal-molasses-yeast-agar-Tegosept medium at 25°C. Unless otherwise noted, all mutations and chromosome aberrations are described in Lindsley and Zimm (1992). osa^1 and osa^2 are EMS-induced alleles (Kennison and Tamkun, 1988). osa^5 and osa^6 are from P-M hybrid dysgenesis between Oregon R and Π2 (J. Kennison, unpublished). The osa^{13} insertion is *l(3)00090* (Karpen and Spradling, 1992; Spradling et al., 1995), a P*[ry, lacZ]* insertion at polytene chromosome bands 90C5-90C8 (STS= Dm0253, BDGP Project Members, 1994, Berkeley *Drosophila* Genome Project). osa^{11} and osa^{12} are EMS-induced alleles recovered as heterozygotes with brm^2 on the basis of the wings-out phenotype (this work). *Df(3R)RD31* is a deficiency that uncovers 89E to 90D (Hopmann et al., 1995) and is osa^-. brm^2 is a null allele (Elfring et al., 1998). The brm^+ rescue transposon $P[w^+, brm^+]$ (=$P[w^+$, *BR14.4]*) and the iso1 line are described by Brizuela et al. (1994).

Genetic interactions

osa, *brm*, and *Antp* interactions were examined by crossing stocks with the appropriate alleles at 25°C. The held-out wings phenotype was scored as flies with one or two wings extended. Interactions of *osa* alleles with $Antp^{Ns}$, $Antp^{73b}$, and the *Hsp70-Antp* transgene were carried out as previously described for *brm* and *mor* (Brizuela and Kennison, 1997; Tamkun et al., 1992).

Isolation of DNA from the *osa* locus

DNA from the *osa* locus was first isolated from the osa^6 line. From a strain that had approximately 12 P-element insertions, the insertion at the *osa* locus was identified by comparing Southern blots of osa^6 with several osa^6 revertant lines. The relevant fragments were subcloned. The cytological location of the clones was determined by in situ hybridization to polytene chromosomes (Engels et al., 1986) to check their 90BC localization. To initiate a chromosome walk through the *osa* region, a 6.5 kb *Sal*I fragment from the iso1 strain (probe 1 in Fig. 4B,C) was used to screen the iso1 r1 genomic cosmid library (Tamkun et al., 1992). Mapping of the chromosomal region was done by Southern blotting, walking with probes purified from different cosmids. Several DNA fragments from the chromosome walk were also in situ hybridized to polytene chromosomes to confirm their 90BC localization.

Nucleic acids analyses

RNAs and DNAs were transferred from agarose gels to HybondN$^+$ membranes according to the manufacturer's instructions. Purified fragments used as probes were [^{32}P]dCTP labeled by random primer with the Random Primer Extension labeling System provided by New England Nuclear, Dupont (Cat No. NEP-103) or the Prime-It II Kit from Stratagene (Cat No. 300385).

Southern blots were done according to standard procedures and were hybridized and washed under stringent conditions (6× SSC, 5× Denhardt's solution, 0.5 mg/ml herring sperm DNA, 0.5% SDS) at 65°C. After hybridization the blots were washed three times in 0.1× SSC, 0.1% SDS at 65°C and exposed to XAR5 Kodak films.

RNA extraction was done according to standard procedures and poly(A)$^+$ RNA was isolated with batched NEB microcrystalline oligo dT (Cat. no. 1403). The northern blots were prepared from 1% agarose gels in 0.22 M formaldehyde, 1× MOPS, and were run in 1× MOPS buffer pH 5.5-7.0 (0.02 M MOPS, 0.005 M sodium acetate, 0.001 M EDTA) at 125 V. Two μg of poly(A)$^+$ were loaded per lane. Prehybridizations, hybridizations and washings were done under the Southern blot conditions.

cDNA *osa* clones were isolated from D2 λgt10 and D3 λgt11 cDNA libraries prepared with RNA from 0 to 3-hour embryos (Poole et al., 1985) using probe 3, and 27 (see Fig. 4). cDNAs were subcloned in pKS$^-$ vector and sequenced with the sequenase version 2.0 DNA sequencing kit (USB, Amersham Cat. No. 70770) using T3, T7, reverse, KS and SK, -40 and -20 primers. Our sequenced cDNAs were from the 5′ end covering exon 1, intron 1, and exon 2, ending at amino acid residue 265 of the published OSA/ELD sequence (Treisman et al., 1997).

The OSA sequence was analyzed with the BLAST program version

2.0 filtered for low complexity and short repetitive sequences (Altschul et al., 1997) obtained from the National Center for Biotechnology Information (NCBI).

Determination of the insertion sites of *osa*^{6} and *osa*^{13}

To determine the P-element insertion sites of these lines, most of the probes from the chromosome walk were hybridized to genomic Southern blots of *osa*^{5}, *osa*^{6}, and *osa*^{13}. We were not able to detect any variation in *osa*^{5}, but we did detect the *osa*^{6} and *osa*^{13} insertion sites. Probe 21 detected both *osa*^{13} (P1486) and *osa*^{6} insertions. More accurate localization of these sites was done. The P1486 (*osa*^{13}) sequence tagged site (STS) (BDGP Project Members, 1994, Berkeley *Drosophila* Genome Project) was determined by nucleotide sequencing and lies inside probe 21, 300 nucleotides from the first methionine of the *osa* gene. The *osa*^{6} insertion site was mapped by amplification with PCR using combinations of 5′ and 3′ end P element primers and a 5′ end primer from the *osa* coding region. The 5′ end primer sequence was 5′GGGACTTTTCACCAAGGCTCC3′ and the 3′ end primer sequence was 5′CCCCACGGACATGCTAAGGG3′. The OSA 5′ primer sequence was 5′ACCGGCCTGCTGTTGC-TGAG3′. Amplification was successful only with the 3′ end P-element and the OSA 5′ primers and the conditions were 95°C, 5 minutes as hot start; 95°C, 1 minute; 65°C, 1 minute; 72°C, 2 minutes; 35 cycles. The *osa*^{6} insertion is 780 nucleotides from the first OSA methionine.

Germ-line clones

To make germline clones, two strategies were followed. In the first *w; TM6C/P[FRT] osa*^{1} or *w;TM6C/P[FRT]osa*^{2} females were crossed to *w; red e/P[w*$^{+}$*, ovo*^{D1}*]*^{C13X2} males (Chou et al., 1993). First instar larvae were irradiated with 1000 rads of gamma rays from a ^{137}Cs source. *w; P[FRT] osa*^{1}*/P[w*$^{+}$*,ovo*^{D1}*]*^{C13X2} or *w;P[FRT]osa*^{2}*/P[w*$^{+}$*,ovo*^{D1}*]*^{C13X2} daughters were collected and crossed to *w; TM6C/P[FRT]osa*^{2} or *w;TM6C/P[FRT]osa*^{1} males, respectively. In the second strategy we used the FLP-FRT method as described in Treisman et al. (1997). The results with the FRT germline analysis did not differ from the radiation-induced clones (data not shown), except that only recombination events proximal to *osa* were induced.

RESULTS

osa interacts genetically with *brm*

osa and *brm* were first identified as suppressors of both the antenna to leg transformation caused by the *Nasobemia* (*Ns*) allele of *Antp* and the extra sex combs phenotype caused by derepression of *Sex combs reduced* (*Scr*) in *Polycomb* (*Pc*) mutants (Kennison and Tamkun, 1988). While examining genetic interactions among trxG mutations, we noted that flies heterozygous for both *brm* and *osa* mutations had a phenotype rarely seen in flies heterozygous for either mutation alone. This phenotype, a held-out wings phenotype, is shown in Fig. 1. We observed the same phenotype with a variety of different *brm* and *osa* alleles (*brm*^{1}, *brm*^{2}, *brm*^{5}, *osa*^{1}, *osa*^{2}, *osa*^{3}, *osa*^{4}, *osa*^{5}, and *osa*^{6}), as well as with deficiencies that include either *brm* [*Df(3L)brm11* and *Df(3L)th102*] or *osa* [*Df(3R)RD31*]. The expressivity of the held-out wings phenotype is more severe in combinations of *brm*^{2} with some point mutations in *osa* (*osa*^{1} and *osa*^{2}) than it is with the *osa* deficiency (Table 1), suggesting that the *osa* point mutations make altered proteins that still bind to something in competition with wild-type OSA proteins, but then fail to function. In a screen to identify mutations that interact genetically with *brm* mutations, we recovered two new *osa* mutations, *osa*^{11} and *osa*^{12}. Both mutations show the same held-out wings phenotype in combination with *brm*^{2}. In the search for mutations that have the held-out wings phenotype when in combination with the *brm*^{2} allele, we did not recover alleles of any other known trxG genes. Meiotic recombination mapping of the factors on the *brm*^{2} and *osa*^{2} chromosomes that interact to give the held-out wings phenotype are consistent with the map positions of *brm* and *osa*. Finally, we tested the effect of *brm* dosage on the held-out wings phenotype. We crossed a *brm*$^{+}$ transgene (Brizuela et al., 1994) into a *brm*^{2} strain and crossed it to a strain carrying

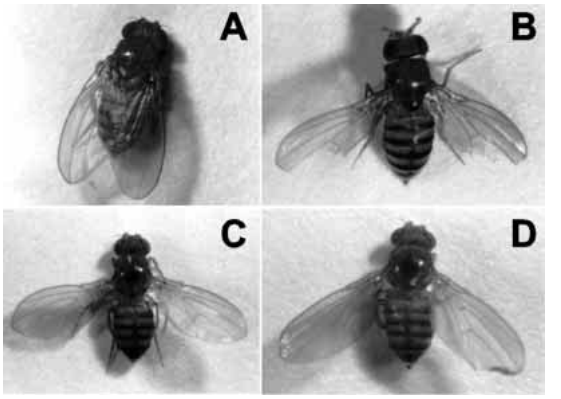

Fig. 1. Flies with the held-out wings phenotype. Representative individuals with the following phenotypes: (A) *brm*^{2}*/TM6C*, (B) *In(3R)Antp*^{B}*/Antp*^{1}, (C) *brm*^{2} *osa*^{2}/+, (D) *brm*^{2} *osa*^{2}/+. The penetrance of these phenotypes is indicated in Table 1.

Table 1. Interactions of brm and osa alleles

Genotype	Flies with held-out wings/Total	Penetrance
Antp^{1}*/In(3R)Antp*^{R}	11/98	11%
Antp^{1}*/In(3R)Antp*^{B}	44/76	58%
brm^{2} *Antp*^{1}*/In(3R)Antp*^{B}	77/80	96%
Antp^{23}*/In(3R)Antp*^{R}	13/94	14%
Antp^{23}*/In(3R)Antp*^{B}	94/135	70%
brm^{2} *Antp*^{23}*/In(3R)Antp*^{B}	75/77	97%
brm^{2}/+	9/498	2%
osa^{2}/+	2/123	2%
brm^{2} *osa*^{2}/+	252/259	97%
osa^{11}/+	1/88	1%
brm^{2}*/osa*^{11}	12/35	35%
brm^{2} *Antp*^{1}*/osa*^{11}	47/98	48%
brm^{2} *Antp*^{23}*/osa*^{11}	31/60	52%
osa^{1}*/brm*^{2}*;brm*$^{+}$*	15/19	79%
osa^{1}*/brm*^{2}‡	52/52	100%
brm^{2} *ash1*^{6}/+	12/500	2%
brm^{2}*trx*^{E2}/+	13/589	2%
brm^{2}*/Trl*^{62}	0/95	0%
brm^{2}*/mor*^{1}	36/151	24%
brm^{2}*/mor*^{2}	23/102	23%

Genotype	Fifth abdominal segmen to fourth/Total	Penetrance
brm^{2}*trx*^{E2}/+	77/142	54%
brm^{2}*ash1*^{6}/+	5/96	5%
brm^{2}*osa*^{2}/+	0/76	0%
brm^{2}*/osa*^{1}	0/32	0%

*The transgenic line used for this cross was w; *brm*^{2}*/TM6C*; P[*w*$^{+}$*brm*$^{+}$]/+. Transgenic virgin females were crossed to *osa*^{1}*/TM6C* males.

‡These are Sb^{+}, *w* males that have the genotype *w*; *brm*^{2} */osa*^{1} from the same cross noted in * using the transgenic line.

osa^{2}. Thus, in this cross we had individuals heterozygous for *osa*^{2} with either one, two, or three copies of *brm*. Direct comparisons of flies of different *brm* gene dosages in the same cross showed that increasing the dosage of wild-type *brm* reduced the held-out wings phenotype as expected (Table 1).

The held-out wings phenotype is not rare in *Drosophila*. It is caused by mutations in many other genes, including *dpp* (*Tg* and *disk-ho* phenotypes) and *Act88F*^{8} (Lindsley and Zimm, 1992). This phenotype was also observed in flies trans-heterozygous for partially complementing *brm* alleles (Brizuela et al., 1994). Nevertheless, the interaction between *brm* and *osa* alleles is unusual because it results from the failure of complementation between mutations in two different genes (non-allelic non-complementation). Although a few other trxG mutations have been shown to interact in double heterozygotes (Dingwall et al., 1995; Tripoulas et al., 1994, 1996), the penetrance in every other case is far less than that observed for the *brm/osa* interactions. In fact, the majority of trxG mutations show little if any interaction in double heterozygotes. *brm* interacts with the trxG genes *trx* and *ash1* to cause partial transformation of the fifth abdominal segment to fourth and metathorax to mesothorax (Dingwall et al., 1995; Tripoulas et al., 1994, 1996; Table 1) but these flies do not hold their wings out at any significantly higher frequency (Table1). We examined flies heterozyogous for *brm*^{2} and a variety of trxG mutations. Less than 3% of flies heterozygous for *brm*^{2} and any of the trxG mutations *ash1*^{6}, *trx*^{E2}, *Trl*^{62}, *Trl*^{3}, *snr*^{P1}, *skd*^{2}, *skd* ^{3}, *dev* ^{1}, *dev* ^{2}, *l(3)87Ca* ^{16}, *kto*^{3}, *kis* ^{1} or *kis*^{2} held their wings out (Table 1 and data not shown). Both *mor*^{1} and *mor*^{2} show some interaction with *brm*^{2} (slightly greater than 20% of transheterozygotes hold their wings out, Table 1).

We next wanted to investigate the basis for the held-out wings phenotype in the *brm/osa* transheterozygotes. The *Antp* gene has two alternative promoters, P1 and P2 (Fig. 2). Genetic studies (Abbott and Kaufman, 1986) showed that the functions of both promoters are essential. Two mutations that inactivate only the P2 promoter have been described, *Antp*^{1} and *Antp*^{23}. The *Antp*^{23} mutation is associated with the insertion of a repetitive element near the P2 promoter (Scott et al., 1983). The precise molecular nature of the *Antp*^{1} mutation is not known, however, there appears to be some alteration in the genomic DNA between the *Hin*dIII site immediately upstream of the P2 transcription start and the *Eco*RI site within the first exon of the P2 transcription unit, probably due to an insertion event (Talbert and Garber, 1994). Chromosome aberrations with breakpoints between the P1 and P2 promoters (shown in Fig. 2) remove P1 function with little or no effect on P2 function (Abbott and Kaufman, 1986). We examined flies heterozygous for the P2-specific mutations (*Antp*^{1} or *Antp*^{23}) and the chromosome aberrations that remove P1 function (Fig. 2). All combinations appeared wild type except flies carrying either *In(3R)Antp*^{R} or *In(3R)Antp*^{B} in combination with the P2-specific mutations. Many of these flies had a held-out wings phenotype indistinguishable from the held-out wings phenotype of the *brm*/*osa* transheterozygotes (Fig. 1). The results are shown in Table 1. While only a few flies with *In(3R)Antp*^{R} show the held-out wings phenotype, the penetrance in flies carrrying *In(3R)Antp*^{B} (the breakpoint closest to the P2 promoter) is much higher, suggesting that disruption of P2 promoter activity can result in a held-out wings phenotype. Moreover, when a *brm*^{2} mutation is introduced, there is a significant increase (P<0.01 for both *Antp*^{1} and *Antp*^{23} by a χ^2 test for homogeneity) in the penetrance of the held-out wings phenotype (Table 1). These results strongly suggest that *brm* is one of the factors required for normal expression of the P2 promoter to prevent the held-out wings phenotype.

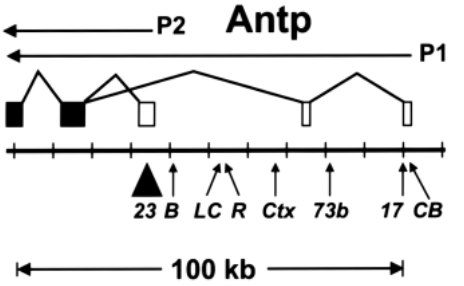

Fig. 2. *Antp* gene structure and chromosome aberrations. In this map the centromere is to the left and the telomere is to the right. P1 and P2 promoters are indicated at the top of the figure with arrows showing transcription initiation from each promoter. White boxes are exons 1-3 not shared among P1 or P2 transcripts. Black boxes are exons 4-8 shared by both transcripts (the smaller introns are not shown). The breakpoints of chromosome aberrations upstream of the P2 promoter and used in this study are shown by vertical arrows with the *Antp* allele designation below (Lindsley and Zimm, 1992). *Antp*^{R}, *Antp*^{B}, *Antp*^{73b}, *Antp*^{LC} and *Antp*^{CB} are inversions. *Antp*^{Ctx} is a transposition and *Antp*^{17} is a translocation. The *Antp*^{23} insertion point is indicated by a black triangle. Scale in the map is 10 kb for every vertical line and the whole region spans 100 kb.

That both *brm* and *osa* are required for activation of the *Antp* P2 promoter is also suggested by their interaction with the *Antp*^{Ns} mutation. The *Antp*^{Ns} mutant chromosome has a large insertion (including a second copy of part of the P2 promoter) upstream of the P2 promoter (Talbert and Garber, 1994). This insertion derepresses the P2 promoter and causes the antennae to differentiate leg structures. The first alleles of both *brm* (*brm*^{1}) and *osa* (*osa*^{1}, *osa*^{2} and *osa*^{3}) were isolated because they failed to derepress the P2 promoter in the *Antp*^{Ns} mutant (Kennison and Tamkun, 1988).

The *osa* gene is an upstream activator of homeotic gene expression

As noted by Kennison and Tamkun (1988) the trxG genes identified in their screen, including the *osa* gene, might regulate HOM gene function at a variety of different levels. They might regulate transcription or translation of the HOM genes, or encode cofactors that interact with the HOM proteins in regulating target genes. Since *brm* has been shown to affect HOM gene transcription (Tamkun et al., 1992), the genetic interaction with *brm* suggests that *osa* may also act at the level of HOM gene transcription. To demonstrate this, we used the same approach that we have previously used for both *brm* (Tamkun et al., 1992) and *moira* (*mor*) (Brizuela and Kennison, 1997).

ANTP proteins are normally not expressed in the cells that form the adult antenna. Misexpression of ANTP proteins during the larval stage in these cells causes them to differentiate leg structures instead of antennal structures. The same mutant phenotype is observed regardless of the promoter used to drive the ectopic expression of ANTP proteins. We

Table 2. *osa* mutations cause a decrease in the penetrance of the antenna to leg transformation in $Antp^{Ns}$, but not $Antp^{73b}$ or *Hsp70-Antp*

Genotype	Transformed flies/Total*	Penetrance
$+/Antp^{Ns}$	216/225	96%
$l(3)72Ab^{1}/Antp^{Ns}$	101/102	99%
$osa^{1}/Antp^{Ns}$	11/204	5%
$osa^{2}/Antp^{Ns}$	17/207	8%
$+/Antp^{73b}$	180/180	100%
$l(3)72Ab^{1}/Antp^{73b}$	63/63	100%
$osa^{1}/Antp^{73b}$	50/50	100%
$osa^{2}/Antp^{73b}$	66/66	100%

Hsp70-Antp flies	Internal control‡	Mutant	Ratio§
$l(3)72Ab^{1}$	51/134 (38%)	34/122 (28%)	0.7
osa^{1}	54/96 (56%)	31/65 (48%)	0.8
osa^{2}	50/116 (43%)	24/68 (35%)	0.8

$Antp^{Ns}$ contains a mutation in the *Antp* P2 promoter. $Antp^{73b}$ is $In(3R)Antp^{73b}$, an inversion that fuses the *sas* promoter to the *Antp* coding sequence. *Hsp70-Antp* is a transgene (see Materials and Methods) with the *Antennapedia* cDNA fused to the *Hsp70* gene promoter. $l(3)72Ab^{1}$, a randomly selected lethal mutation, was used as a control.

*The number of flies with transformed tissue/number of flies scored. The transformation scored was the appearance of leg tissue in the antennae or aristae.

‡The internal control are flies in the same vial that carried the *TM6C* balancer chromosome instead of the *osa* or $l(3)72Ab^{1}$ mutations.

§The ratio is the penetrance in the flies with the *osa* or $l(3)72Ab^{1}$ mutations divided by the penetrance in the internal control.

have used three different promoters (the *Antp* P2 promoter, the *sas* promoter, and the *Hsp70* promoter) to drive ectopic expression of ANTP proteins in the imaginal antennal cells. As described above, the $Antp^{Ns}$ allele derepresses the *Antp* P2 promoter in the eye-antennal disc, expressing wild-type *Antp* transcripts from the *Antp* promoter (Jorgensen and Garber, 1987; Talbert and Garber, 1994). In contrast, the $Antp^{73b}$ inversion breaks within the *Antp* gene and expresses a hybrid transcript in the imaginal antennal cells under the control of the *sas* promoter (Frischer et al., 1986; Schneuwly et al., 1987a). This hybrid transcript includes the 3′ *Antp* exons that contain the open reading frame for the ANTP proteins. Thus, the $Antp^{73b}$ mutation produces ANTP proteins in the imaginal antennal cells from a foreign promoter, while the $Antp^{Ns}$ mutation produces ectopic ANTP proteins in the same cells under the control of an *Antp* promoter. In both cases, the same mutant phenotype results. As shown in Table 2 the penetrance of the antenna-to-leg transformation of $Antp^{Ns}$ mutants is greatly reduced in osa^{1} and osa^{2} heterozygotes (from 96% to 5% and 8% for osa^{1} and osa^{2}, respectively). In contrast, osa^{1} and osa^{2} do not affect either the penetrance or expressivity of the $Antp^{73b}$ mutant phenotype (Table 2). These data are similar to those with both *brm* (Tamkun et al., 1992) and *mor* (Brizuela and Kennison, 1997). Finally, we have also used a mutant strain that contains a transgene with the heat-inducible *Hsp70* promoter fused to an *Antp* cDNA (Tamkun et al., 1992). This transgenic strain expresses ANTP proteins ubiquitously when heat shocked, with heat shocks at the appropriate time during larval growth causing antennal to leg transformations (Gibson and Gehring, 1988; Schneuwly et al., 1987b). As shown in Table 2, *osa* mutations do not affect the phenotype caused by ectopic expression of ANTP proteins from the *Hsp70* promoter. Together, these results show that high levels of *osa* expression are required only for the *Antp* P2 promoter, and not for the function of ANTP proteins expressed from either the *sas* or *Hsp70* promoters.

osa is required maternally for proper embryonic segmentation

Although *osa* function appears to be important for expression of some HOM and segmentation genes in imaginal tissues, homozygous *osa* mutants die late in embryogenesis with no clear defects in either segmentation or segment identity. To determine whether wild-type maternal *osa* gene products deposited in the egg might be sufficient for segmentation and segment identity, we generated homozygous germ cells for the osa^{1} or osa^{2} alleles, which are strong $Antp^{Ns}$ suppressors. We used mitotic recombination and a transgene carrying the dominant female-sterile mutation ovo^{D1} (Chou et al., 1993) to produce embryos that lack wild-type maternal *osa* functions. Using a transposon with the ovo^{D1} allele inserted in 3R (Chou et al., 1993) we have used both radiation-induced mitotic recombination (for both osa^{1} and osa^{2}) and FRT-FLP mediated recombination (for osa^{2}) (Golic and Lindquist, 1989) to remove maternal *osa* functions. In all cases, the results were identical and are shown in Fig. 3. Loss of maternal *osa* functions has dramatic effects on segmentation of the embryo. When rescued by a wild-type allele inherited from the father, the embryos secrete cuticle but have severe defects in segmentation, resembling mutants for the early-acting gap segmentation genes. When both the maternal and zygotic *osa* functions are lacking, the embryos fail to differentiate cuticle at all. The failure to detect obvious changes in the homozygous *osa* mutants from heterozygous mothers is clearly a consequence of the maternally encoded *osa* gene products functioning early in embryogenesis to activate transcription of target genes. Because of the severe defects in the embryos lacking maternal *osa* functions and the cascade of regulatory interactions between the segmentation and HOM genes early in embryogenesis, we have not tried to identify the earliest-acting genes affected by loss of *osa* function.

Molecular analysis of *osa*

To begin the molecular analysis of the *osa* gene we took advantage of a P-element inserted in the *osa* locus in the osa^{6} mutant strain. Once we had identified the P-element inserted in the *osa* locus (see Materials and Methods), DNA from this strain was digested with *Hin*dIII and used to construct a genomic DNA library. A 3.6 kb hybrid genomic P-element fragment was recovered from this library and used to screen an iso1 genomic DNA library to begin a chromosome walk in the 90BC region of the third chromosome (*osa* maps between salivary chromosome bands 90B1 and 90D1, (Kennison and Tamkun, 1988). We recovered and analyzed about 65 kb of genomic DNA from the 90BC salivary gland chromosome region surrounding the P-element insertion in the osa^{6} strain. This region is shown in Fig. 4A. Another P-element insertion that also fails to complement all *osa* mutations maps near the osa^{6} insertion site. This second P-element insertion, *l(3)00090* or P1486, has been localized to 90C1-2 (Treisman et al., 1997). We will refer to this second P-element insertion as osa^{13}. We mapped both P-element insertions very close to one another in

the same 3.0 kb *Pst*I fragment as shown in Fig. 4A by Southern experiments and more accurately by nucleotide sequencing and PCR (data not shown, see Methods). osa^6 and osa^{13} are at 780 and 300 nucleotides, respectively, from the first methionine of the *osa* gene. Both insertions map in the first intron between a non-coding first exon and the first coding exon of the *osa* gene, in agreement with Treisman et al. (1997). The osa^6 element is inserted in the direction of *osa* transcription and the osa^{13} P element is inserted in the opposite direction (Fig. 4A, see Methods).

osa encodes a putative DNA-binding protein

To find the *osa* transcription unit, we characterized the mRNAs from the genes present in this genomic region. We did northern analyses of poly(A)$^+$ RNA from 3 to 12 and 12 to 24-hour embryos, using as probes, fragments spanning the 65 kb genomic region of our walk. The results are shown in Fig. 4B,C. In particular we found that probes from the genomic region covered between probes 11 and 3 (Fig. 4B) detected a 10 kb mRNA that is present in embryos, larvae, and early pupae (Fig. 4D). The osa^6 and osa^{13} insertion sites appear to be near or within this transcription unit. We screened a 0-3 hour embryonic cDNA library and began sequencing cDNA clones corresponding to the 10 kb transcript that we detected on northern blots. In the course of this study, Treisman et al. (1997) published the entire sequence of a 10 kb transcript surrounding the osa^{13} [*l(3)00090*] insertion. Although they described the insertion as identifying a novel gene that they have named *eyelid* (*eld*), it is clear from the mutant phenotypes and the failure to complement *osa* mutations that *eld* is identical to the previously described *osa* gene (Kennison and Tamkun, 1988). Comparisons of the restriction endonuclease sites in the genomic map and our partial cDNA sequence confirm that *eld* and *osa* are the same gene. Our genomic maps are identical for the *Eco*RI and *Not*I sites with the exception of two *Eco*RI bands of 2.0 kb and 0.8 kb, that Treisman et al. (1997) described as intronic sequences but which we do not detect.

Treisman et al. (1997) showed that *osa* (*eld*) encodes a large nuclear protein (the ORF has the potential to encode a protein of 2713 amino acids) that is ubiquitously expressed in the early embryo. The OSA protein contains a region of homology to the DNA-binding domains of the *Drosophila* DEAD-RINGER (Gregory et al., 1996) and mouse BRIGHT (Herrscher et al., 1995) proteins.

The DEAD-RINGER (DRI) protein was identified in a screen for proteins that bound a fragment of DNA with the consensus sequence for binding of the *Drosophila* ENGRAILED (EN) homeodomain (Kalionis and O'Farrell, 1993). Most of the clones found in this analysis were homeodomain proteins, but the DRI protein that lacks any homology to the homeodomain was also identified (Gregory et al., 1996). In light of the findings that both *osa* and *en* are upstream regulators of homeotic genes (this work, Bienz et al., 1988 and Boulet and Scott, 1988) and in particular that *osa* together with *brm* could regulate a response element at the *Antp* P2 promoter, it is very interesting that the OSA protein might bind DNA at sequences recognized by homeodomain-containing proteins.

DISCUSSION

Requirement of OSA and other trx group proteins for expression of the *Antp* P2 promoter and other target genes

We have characterized another member of the trx group, *osa*. The *osa* gene was first identified as a suppressor of the $Antp^{Ns}$ mutation, as well as a suppressor of the extra sex combs phenotype caused by derepression of *Scr* in *Pc* mutants. The $Antp^{Ns}$ mutation has been molecularly characterized and is complex (Talbert and Garber, 1994). The wild-type *Antp* gene has two promoters, P1 and P2. The $Antp^{Ns}$ mutant chromosome carries a duplication of part of the P2 promoter as part of a large insertion that also includes DNA of unknown origin. In this mutant chromosome, P1 and P2 are weakly and strongly derepressed, respectively, in the eye-antennal imaginal disc. As

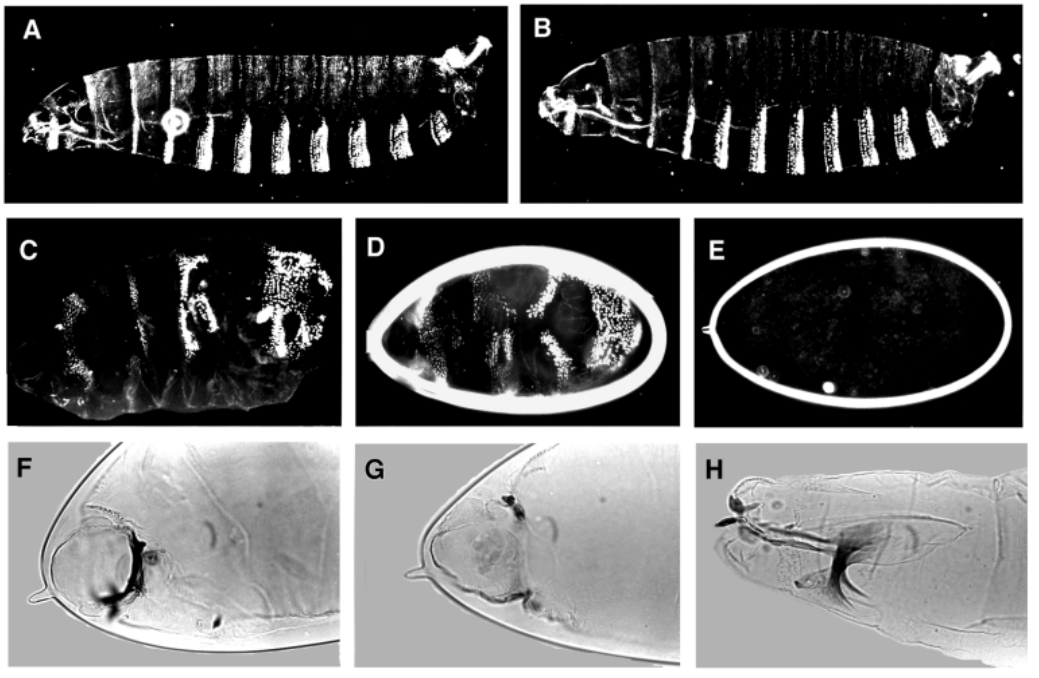

Fig. 3. Embryonic phenotype of maternal and zygotic *osa* mutants. In all specimens shown anterior is to the left and posterior to the right. Cuticle preparations of wild-type (A) and osa^- (B) late embryos derived from heterozygote *osa* parents (osa^1/*TM6C* X osa^2/*TM6C*) (lateral views). Ventral view of osa^1 (C) and osa^2 (D) germline clone-derived embryos with paternal rescue. (F,G), side views of mouth parts of embryos of type C (osa^1/+) and D (osa^2/+) respectively; (H) wild type. (E) osa^1 germline clone-derived zygote without paternal rescue.

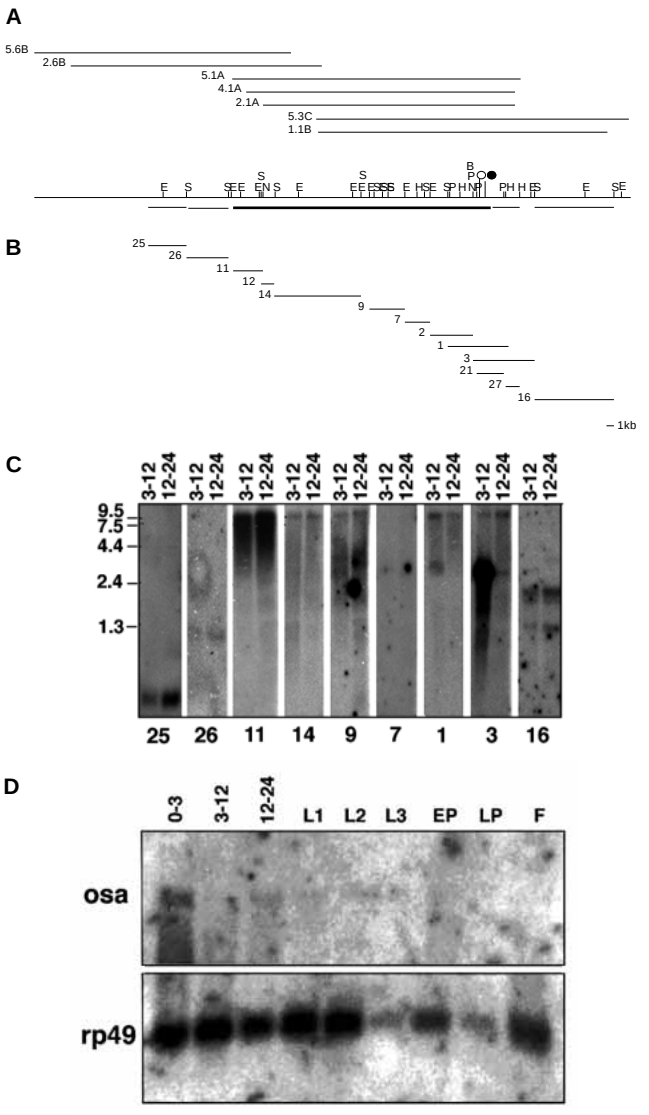

Fig. 4. Molecular map and developmental expression of transcripts in the *osa* region. (A) Representative cosmids isolated with probe 1 (shown in C, see Methods) used to walk through the *osa* region. The restriction map of the region is shown below the cosmids (S, *Sal*I; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; B, *Bam*HI; N, *Not*I). Only some H, P and B sites are shown. The circles show the insertion sites of P elements in the osa^{13} (white circle) and osa^{6} (black circle) lines, determined by genomic Southern blotting, nucleotide sequencing, and PCR (see Methods). Horizontal lines just under the genomic map represent the regions that detect the different mRNAs shown in C. Of these regions the bold line shows the genomic region covered by the *osa* transcript. (B) Genomic probes used to detect the poly(A)$^+$ mRNA from 3 to 12-hour and 12 to 24-hour embryos encoded in the *osa* region. All the probes except probe 1 (see Material and Methods) were isolated from cosmids shown in A. (C) Transcripts of the *osa* region. Northern blottings with poly(A)$^+$ mRNA isolated from Oregon R 3 to 12 and 12 to 24-hour embryos. Probes that detect the 10 kb *osa* mRNA are 11,14, 9, 1 and 3; other transcripts in the region are a 470 bp transcript (probe 25); a 1.3 kb (probe 26); a 2.8 kb transcript (probe 3) and at least two transcripts one of 2.0 and another one of 1.3 kb, (probe 16). (D) Developmental northern blot of *osa* mRNA. In the upper part of the figure are indicated the stages from which the poly(A)$^+$ RNA was purified: embryos from 0 to 3, 3 to 12- and 12 to 24-hour; 1st (L1), 2nd (L2), and 3rd (L3) instar larvae; early pupae (EP); late pupae (LP); adult females (F). 2 μg of poly(A)$^+$ RNA were loaded per lane. The blot was hybridized sequentially against probe 3 (B) to detect the *osa* mRNA (upper part) and with a probe for the ribosomal protein rp49 mRNA (0.6kb) as a loading control (lower part). Molecular weight markers are shown on the left.

P2 is much more strongly derepressed, it is probably responsible for most of the mutant phenotype. Mutations in *osa* or *brm* strongly suppress the $Antp^{Ns}$ mutant phenotype.

brm and *osa* interact genetically to give a held-out wings phenotype. This is another phenotype that can result from a failure to properly activate the *Antp* P2 promoter. As lowered dosage of *brm* enhances the held-out wings phenotype in the *Antp* P2 partial loss of function ($Antp^{B}/Antp^{1}$ and $Antp^{B}/Antp^{23}$ genotypes), it is likely that the held-out wings phenotype of the *brm*/*osa* transheterozygous flies is due to a failure to completely activate the *Antp* P2 promoter. The difference in phenotype between $In(3R)Antp^{B}$ and $In(3R)Antp^{R}$ in combination with the P2 promoter-specific mutations indicates that there is a *cis*-regulatory element between the two breakpoints that requires high levels of BRM and OSA proteins in order to activate transcription.

Though the trxG of genes is potentially heterogeneous with regard to the nature of the steps that potentially can influentiate HOM expression (Kennison and Tamkun, 1988), several have been shown to affect transcription, including *brm, trx, Trithorax-like, mor, ash1, ash2,* and *E(var)93D* (Brizuela and Kennison, 1997 and references therein). All these genes are pleiotropic in that they are not only required for HOM gene expression, but are also required for the expression of other genes as well. For example, both *brm* and *mor* are required for oogenesis (Brizuela et al., 1994; Brizuela and Kennison, 1997), while none of the HOM genes are required.

We found that *osa* is also a pleiotropic regulator of transcription. With the *osa* germ-line clones, as for other members of the trxG like *trx* (Ingham, 1983, 1985), *brm* (Brizuela et al., 1994), and *mor* (Brizuela and Kennison, 1997), we have shown that there are target genes in addition to the homeotic genes. Nevertheless the germline clones for *trx*, *brm*, *mor* and *osa* are not identical. In contrast to *brm* and *mor,* we found that *osa* is not required for oogenesis. *brm* is germline lethal (*brm* clones do not make eggs) (Brizuela et al., 1994) while *osa* clones make normal eggs that develop only to embryonic stages no matter what the zygotic genotype (this work). *mor* clones make abnormal eggs that are not fertilized (Brizuela and Kennison, 1997). *trx* clones make normal eggs that can be rescued by wild-type sperm to develop to adults (Ingham, 1983, 1985). There is no paternal rescue for loss of maternal *brm* products (Brizuela et al., 1994), but there is for *osa* (data in this paper).

Treisman et al. (1997) described *eld* as an indirect antagonist of the wingless (WG) pathway, but were unable to determine the nature of the antagonism. They suggested that ELD functions as a repressor downstream of WG in the signalling pathway. As we have shown here, *eld* is actually allelic to *osa*. Although OSA appears to be an activator for all of the target genes that we have examined, it is possible that it can also act as a repressor. For example, it could act with the BRM complex to facilitate binding of repressors as well as activators to target sites in regulated genes. It is also possible that OSA represses

indirectly by activating transcription of a gene encoding a repressor. Given the pleiotropic phenotypes of *osa* mutants, there are probably many different target genes that require OSA proteins for transcription. One of these must be involved in the wingless signalling pathway, but none of the genes known to be involved in this pathway is a good candidate for the OSA effector. It is likely that one of the yet undiscovered factors in this pathway mediates the OSA requirement.

The OSA and BRM proteins may act together in transcriptional activation recruiting the BRM complex to some target genes

We have shown here that *osa* is the *eld* gene. Sequencing data (Treisman et al., 1997) reveal that the 2713 amino acid protein is highly repetitive with many tracks of low complexity sequences. Two regions of OSA have homology to other genes. Within region I (residues 854 to 1104) there is a 97 amino-acid sequence (residues 993 to 1087) that contains a putative ARID (AT-rich interaction domain) domain that is conserved in the *Drosophila* DRI and mouse BRIGHT proteins (Gregory et al., 1996; Herrscher et al., 1995) and in at least 10 other proteins. Although the DRI protein was identified in a screen for proteins that bound a consensus sequence for the EN homeodomain (Kalionis and O'Farrell, 1993) it lacks any homology to the homeodomain (Gregory et al., 1996). The BRIGHT (B cell regulator of IgH transcription) protein binds to the minor groove of a consensus MAR (Matrix Attachment Region) sequence. MARs organize chromatin fibers into looped domains by attachment to the nuclear matrix and may function as boundary elements for transcriptional domains (for review see Bode et al., 1996). They may also collaborate with enhancers to generate extended domains of accessible chromatin (Jenuwein et al., 1997). DRI and BRIGHT are sequence-specific DNA binding proteins and the ARID domain is essential, but not sufficient for this binding. The consensus target sequences for DRI, BRIGHT and EN binding are very similar. DRI binds the PuATTAA sequence (Gregory et al., 1996), BRIGHT binds the PuATa/tAA sequence (Herrscher et al., 1995) and EN binds GATCAATTAAAT (NP_6, Desplan et al., 1988). All of these contain the same ATTAA core sequence.

The second region of homology in the OSA protein, region II, extends from residues 1757 to 2509. Extensive filtered BLAST searches (Altschul et al., 1997) (see Methods) with this region did not show any other homology besides the ones previously reported by Treisman et al. (1997), a predicted protein from *Caenorhabditis elegans*, two human expressed sequence tags (ESTs), and ESTs from mouse and rat. Our searches did not show any other motif that might predict the function of this second region, nor any other protein with regions I and II. As noted by Treisman et al. (1997) the two *C. elegans* genes with homologies to the two regions of OSA are adjacent to each other in the genome and might actually be a single gene.

Of the other 10 proteins reported with an ARID domain (Gregory et al., 1996; Herrscher et al., 1995), we are particularly interested in the SWI1 protein, given the fact that it is a member of the SWI/SNF complex. Thus, we investigated the possibility that OSA might be the putative *Drosophila* SWI1 homolog. SWI1 has long tracks of polyasparagine, polyglutamine, and a putative Cys_4 zinc-finger motif (O'Hara et al., 1988). OSA is very rich in proline but no zinc-finger motif was detected. SWI1 has in common with OSA clusters of sequence made up principally of only two or three amino acids. Very recently, a protein called p270 has been described as a member of the human BRG1 complex and has been proposed as a human SWI1 homolog (Dallas et al., 1998). p270, like OSA and SWI1, has glutamine-rich regions, an ARID domain and several copies of the LXXLL motif (where L is leucine and X is any amino acid). This motif mediates binding to nuclear receptors (Heery et al., 1997). Interestingly, OSA has three copies of this motif. Although *Drosophila* ESTs corresponding to proteins related to several yeast SWI/SNF subunits (including SWI2/SNF2, SWI3, SNF5, and SWP73) have been recovered, it is interesting to note that no EST corresponding to SWI1 has yet been identified. It is possible that OSA, SWI1 and p270 ARID-domain-containing proteins play similar roles in their respective organisms.

Is OSA essential for the function of the BRM complex? If so, we would expect *brm* and *osa* mutants to have identical phenotypes, and the mutation with the strongest effects in one assay should be the mutation with the stronger effects in all other assays. This is not what we observe. For example there are much greater effects on *Scr*, *Ubx*, and *Abd-B* in *brm* heterozygotes than in *osa* heterozygotes, but the reverse is observed for *Antp* (Kennison and Tamkun, 1988; Tamkun et al., 1992; J. Kennison, unpublished results). Another important difference is the germ line requirements for *brm* and *osa*, i. e., *brm* clones do not make eggs (Brizuela et al., 1994) while *osa* clones make normal appearing eggs that are fertilized but fail during embryogenesis (Treisman et al., 1997 and this work). Thus, *brm* is required under conditions that do not appear to require *osa*. If OSA is a subunit of the BRM complex, it is not essential for all of the complex's functions. Consistent with this possibility, the OSA protein was not identified as one of the major subunits of the BRM complex in the *Drosophila* embryo (Papoulas et al., 1998). However, it remains possible that OSA is a substoichiometric subunit of the BRM complex, or that it is associated with BRM at other stages of development.

Another possibility is that the OSA protein targets the BRM complex to specific promoters (e. g. *Antp* P2) in the same way that the glucocorticoid receptor (Ostlund-Farrants et al., 1997; Yoshinaga et al., 1992) and other activators (Burns and Peterson, 1997; Utley et al., 1997 and references therein) interact with the SWI/SNF complex. In yeast, the SWI/SNF complex has considerable non-sequence-specific affinity for DNA (Quinn et al., 1996). Quinn et al. showed that the SWI/SNF complex has a DNA-binding activity with characteristics reminiscent of HMG box proteins. Nevertheless, the SWI/SNF complex is required only by a small subset of inducible genes. What is the targeting mechanism to recruit the complex to the required genes? The yeast protein SWI3 has a segment called the SANT (SWI3, ADA2, N-CoR, and TFIIIB B″) domain that has been proposed as a DNA-binding domain (Aasland et al., 1996). It has also been proposed (Carlson and Laurent, 1994; Logie and Peterson, 1997) that SWI/SNF association with DNA-binding transcriptional activators or with the transcription machinery could be used in the targeting mechanism. To date, no protein from the SWI/SNF complex (including SWI3 or the ARID-domain protein SWI1), has been shown to bind DNA in a sequence-specific manner.

We propose that the OSA protein may be involved in the

targeting of the BRM complex in *Drosophila*. Whether an intrinsic member of the BRM complex or merely an associated partner, we believe that the OSA protein may interact with specific target sequences in *cis*-regulatory elements to anchor or recruit the BRM complex. Given the patterns of expression driven by *Antp cis*-regulatory sequences in a reporter gene transposon (Boulet and Scott, 1988), it is likely that there are EN DNA-binding sites in the 10 kb region 5′ to the Antp P2 promoter. Since the ARID domain found in the OSA protein may bind to EN target sites, it is possible that OSA proteins will bind directly to these sequences. It is also possible that OSA may bind AT-rich regions of DNA with little specificity**.** We are very excited at the prospect of identifying putative *brm* and *osa* response elements for in vitro studies. The delineation of *brm* and *osa* response elements should allow us to clarify whether they act in concert or independently. It is also possible that the BRM complex alters chromatin structure in order to facilitate the binding of OSA to its target sites**.** OSA would subsequently act independently of the BRM complex to activate transcription. In the future, we hope to determine whether OSA binds DNA, either in a sequence-specific manner or non-specifically, and whether it is an intrinsic subunit of one or more BRM complexes.

We thank John Tamkun for useful discussions and for critical reading of the manuscript. We thank Dr Ian Duncan and the Bloomington Stock Center for mutant strains, Dr Susan Haynes for kindly providing RNA samples. We thank Susan Haynes, Judy Kassis, Mario Zurita, Renate Deuring, Lisa Elfring, Adam Felsenfeld, Brenda Brizuela, and Carl Wu for help and advice. M. V. at the IBT, UNAM, Mexico acknowledges funds from a PAPIIT grant No. IN200696.

REFERENCES

Aasland, R., Stewart, A. F. and Gibson, T. J. (1996). The SANT domain: a putative DNA binding domain in the SWI/SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB. *Trends Biochem. Sci.* **21,** 87-88.

Abbott, M. K. and Kaufman, T. C. (1986). The relationship between the functional complexity and the molecular organization of the *Antennapedia* locus of *Drosophila melanogaster. Genetics* **114,** 919-942.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25,** 3389-3402.

Bienz, M., Saari, G., Tremml, G., Muller, J., Zust, B. and Lawrence, P. A. (1988). Differential regulation of *Ultrabithorax* in two germ layers of *Drosophila*. *Cell* **53,** 567-576.

Bode, J., Stengert-Iber, M., Kay, V., Schlake, T. and Dietz-Pfeilstetter, A. (1996). Scaffold/matrix-attached regions: topological switches with multiple regulatory functions. *Crit. Rev. Eukaryot. Gene Expr*. **6,** 115-138.

Boulet, A. and Scott, M. P. (1988). Control elements of the P2 promoter of the *Antennapedia* gene. *Genes Dev.* **2,** 1600-1614.

Brizuela, B. J., Elfring, L., Ballard, J., Tamkun, J. W. and Kennison, J. A. (1994). Genetic analysis of the *brahma* gene of *Drosophila melanogaster* and polytene chromosome subdivisions 72AB. *Genetics* **137,** 803-813.

Brizuela, B. J. and Kennison, J. A. (1997). The *Drosophila* homeotic gene *moira* regulates expression of *engrailed* and HOM genes in imaginal tissues. *Mech. Dev.* **65,** 209-220.

Burns, G. L. and Peterson, C. L. (1997). The yeast SWI-SNF complex facilitates binding of a transcriptional activator to nucleosomal sites *in vivo*. *Mol. Cell. Biol.* **17,** 4811-4819.

Cairns, B. R., Kim, Y.-J., Sayer, M. H., Laurent, B. C. and Kornberg, R. D. (1994). A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. *Proc. Natl. Acad. Sci. USA* **91,** 1950-1954.

Cairns, B. R., Levinson, R. S., Yamamoto, K. R. and Kornberg, R. D. (1996a). Essential role of Swp73p in the function of yeast Swi/Snf complex. *Genes Dev.* **10,** 2131-2144.

Cairns, B. R., Henry, L. N. and Kornberg, R. D. (1996b). TFG3/TAF30/ANC1, a component of the yeast SWI/SNF complex that is similar to the leukemogenic proteins ENL and AF-9. *Mol. Cell. Biol.* **16,** 3308-3316.

Carlson, M. and Laurent, B. C. (1994). The SNF/SWI family of global transcriptional activators. *Curr. Opin. Cell. Biol.* **6,** 396-402.

Chou, T. B., Noll, E. and Perrimon, N. (1993). Autosomal P[*ovo*^{D1}] dominant female-sterile insertions in *Drosophila* and their use in generating germ-line chimeras. *Development* **119,** 1359-1369.

Côtè, J., Quinn, J., Workman, J. L. and Peterson, C. L. (1994). Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265,** 53-60.

Dallas, P. B., Cheney, I. W., Liao, D., Bowrin, V., Byam, W., Pacchione, S., Kobayashi, R., Yaciuk, P. and Moran, E. (1998) p300/CREB binding protein-related protein p270 is a component of mammalian SWI/SNF complexes. *Mol. Cell. Biol.* **18,**3596-3603.

Desplan, D., Theis, J. and O'Farrell, P. H. (1988). The sequence specificity of homeodomain-DNA interaction. *Cell* **54,** 1081-1090.

Dingwall, A. K., Beek, S. J., McCallum, C. M., Tamkun, J. W., Kalpana, G. V., Goff, S. P. and Scott, M. P. (1995). The *Drosophila* snr1 and *Brm* proteins are related to yeast SWI/SNF proteins and are components of a large protein complex. *Mol. Biol. Cell* **6,** 777-791.

Duncan, I. M. (1987). The bithorax complex. *Annu. Rev. Genet.* **21,** 285-319.

Elfring, L., Daniel, C., Papoulas, O., Deuring, R., Sarte, M., Moseley, S., Beek, S., Waldrip, R., Daubresse, G., DePace, A., Kennison, J. and Tamkun, J. (1998). Genetic analysis of *brahma*: the *Drosophila* homolog of the yeast chromatin remodeling factor SWI2/SNF2. *Genetics* **148,** 251-265.

Engels, W. R., Preston, C. R., Thompson, P. and Eggleston, W. B. (1986). *In situ* hybridization to *Drosophila* salivary chromosomes with biotinylated DNA probes and alkaline phosphatase. *BRL Focus* **8,** 6-8.

Frischer, L. E., Hagen, F. S. and Garber, R. L. (1986). An inversion that disrupts the *Antennapedia* gene causes abnormal structure and localization of RNAs. *Cell* **47,** 1017-1023.

Gaudreau, L., Schmid, A., Blaschke, D., Ptashne, M. and Hörz, W. (1997). RNA polymerase II holoenzyme recruitment is sufficient to remodel chromatin at the yeast PHO5 promoter. *Cell* **89,** 55-62.

Gibson, G. and Gehring, W. J. (1988). Head and thoracic transformations caused by ectopic expression of *Antennapedia* during *Drosophila* development. *Development* **102,** 657-675.

Golic, K. G. and Lindquist, S. (1989). The FLP recombinase of yeast catalyzes site-specific recombination in the *Drosophila* genome. *Cell* **59,** 499-509.

Gregory, S. L., Kortschak, R. D., Kalionis, B. and Saint, R. (1996). Characterization of the *dead ringer* gene identifies a novel, highly conserved family of sequence-specific DNA-binding proteins. *Mol. Cell. Biol.* **16,** 792-799.

Heery, D. M., Kalkhoven, E., Hoare, S. and Parker, M. G. (1997). A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature* **387,** 733-736.

Herrscher, R. F., Kaplan, M. H., Lelsz, D. L., Das, C., Scheuermann, R. and Tucker, P. W. (1995). The immunoglobulin heavy-chain matrix-associating regions are bound by Bright: a B cell-specific trans-activator that describes a new DNA-binding protein family. *Genes Dev.* **9,** 3067-3082.

Hopmann, R., Duncan, D. and Duncan, I. (1995). Transvection in the *iab-5,6,7* region of the bithorax complex of *Drosophila*: homology independent interactions in trans. *Genetics* **139,** 815-833.

Ingham, P. W. (1983). Differential expression of bithorax complex genes in the absence of the *extra sex combs* and *trithorax* genes. *Nature* **306,** 591-593.

Ingham, P. W. (1985). A clonal analysis of the requirement for the *trithorax* gene in the diversification of segments in *Drosophila*. *J. Embryol. exp. Morph.* **89,** 349-365.

Ingham, P. W. and Martínez Arias, A. (1992). Boundaries and fields in early embryos. *Cell* **68,** 221-235.

Jenuwein, T., Forrester, W. C., Fernández-Herrero, L. A., Laible, G., Dull, M. and Grosschedl, R. (1997). Extension of chromatin accessibility by nuclear matrix attachment regions. *Nature* **385,** 269-272.

Jorgensen, E. M. and Garber, R. L. (1987). Function and misfunction of the two promoters of the *Drosophila Antennapedia* gene. *Genes Dev.* **1,** 544-555.

Kalionis, B. and O'Farrell, P. H. (1993). A universal target sequence is bound *in vitro* by diverse homeodomains. *Mech. Dev.* **43,** 57-70.

Karpen, G. H. and Spradling, A. C. (1992). Analysis of subtelomeric heterochromatin in the *Drosophila* minichromosome Dp1187 by single P element insertional mutagenesis. *Genetics* **132,** 737-753.

Kaufman, T. C., Seeger, M. A. and Olsen, G. (1990). Molecular and genetic organization of the *Antennapedia* gene complex of *Drosophila melanogaster*. *Adv. Genet.* **27,** 309-362.

Kennison, J. A. (1993). Transcriptional activation of *Drosophila* homeotic genes from distant regulatory elements. *Trends Genet.* **9,** 75-79.

Kennison, J. A. (1995). The Polycomb and trithorax group proteins of *Drosophila*: trans-regulators of homeotic gene function. *Ann. Rev. Genet.* **29,** 289-303.

Kennison, J. A. and Tamkun, J. W. (1988). Dosage-dependent modifiers of *Polycomb* and *Antennapedia* mutations in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **85,** 8136-8140.

Kennison, J. A. and Tamkun, J. W. (1992). Trans-regulation of homeotic genes in *Drosophila*. *The New Biologist* **4,** 91-96.

Laurent, B. C. and Carlson, M. (1992). Yeast SNF2/SWI2, SNF5, and SNF6 proteins function coordinately with the gene-specific transcriptional activators GAL4 and Bicoid. *Genes Dev.* **6,** 1707-1715.

Lindsley, D. L. and Zimm, G. G. (1992). The genome of *Drosophila melanogaster*. Academic Press, San Diego, Calif., pp. 1133.

Logie, C. and Peterson, C. L. (1997). Catalytic activity of the yeast SWI/SNF complex on reconstituted nucleosome arrays. *EMBO J.* **16,** 6772-6782.

O'Hara, P. J., Horowitz, H., Eichinger, G. and Young, E. T. (1988). The yeast ADR6 gene encodes homopolymeric amino acid sequences and a potential metal-binding domain. *Nucl. Acids Res.* **16,** 10153-10169.

Ostlund-Farrants, A. K., Blomquist, P., Kwon, H. and Wrange, O. (1997). Glucocorticoid receptor-glucocorticoid response element binding stimulates nucleosome disruption by the SWI/SNF complex. *Mol. Cell. Biol.* **17,** 895-905.

Papoulas, O., Beek, S. J., Moseley, S. L., McCallum, C. M., Sarte, M., Shearn, A. and Tamkun, J. W. (1998). The *Drosophila* trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. *Development* (in press).

Peterson, C. L., Dingwall, A. and Scott, M. P. (1994). Five SWI/SNF gene products are components of a large multisubunit required for transcriptional enhancement. *Proc. Natl. Acad. Sci. USA* **91,** 2905-2908.

Poole, S., Kauvar, L. M., Drees, B. and Kornberg, T. (1985). The *engrailed* locus of *Drosophila*: structural anlysis of an embryonic transcript. *Cell* **40,** 37-43.

Quinn, J., Fryberg, A. M., Ganster, R. W., Schmidt, M. C. and Peterson, C. L. (1996). DNA-binding properties of the yeast SWI/SNF complex. *Nature* **379,** 844-847.

Schneuwly, S., Kuroiwa, A. and Gehring, W. J. (1987a). Molecular analysis of the dominant homeotic Antennapedia phenotype. *EMBO J.* **6,** 201-206.

Schneuwly, S., Klemenz, R. and Gehring, W. J. (1987b). Redesigning the body plan of *Drosophila* by ectopic expression of the homeotic gene *Antennapedia*. *Nature* **325,** 816-818.

Scott, M. P., Weiner, A. J., Hazelrigg, T. I., Polisky, B. A., Pirrotta, V., Scalenghe, F. and Kaufman, T. C. (1983). The molecular organization of the *Antennapedia* locus of *Drosophila*. *Cell* **35,** 763-776.

Simon, J. (1995). Locking in stable states of gene expression: transcriptional control during *Drosophila* development. *Curr. Opin. Cell. Biol.* **7,** 376-385.

Spradling, A. C., Stern, D. M., Kiss, I., Roote, J., Laverty, T. and Rubin, G. M. (1995). Gene disruptions using P transposable elements: An integral component of the *Drosophila* genome project. *Proc. Natl. Acad. Sci. USA* **92,** 10824-10830.

Talbert, P. B. and Garber, R. L. (1994). The *Drosophila* homeotic mutation *Nasobemia* (*Antp^{Ns}*) and its revertants: and analysis of mutational reversion. *Genetics* **138,** 709-720.

Tamkun, J. W., Deuring, R., Scott, M. P., Kissinger, M., Pattatuci, A. M., Kaufman, T. C. and Kennison, J. A. (1992). *brahma*: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* **68,** 561-572.

Treich, I., Cairns, B. R., de los Santos, T., Brewster, E. and M. Carlson, M. (1995). SNF11, a new component of the yeast SNF-SWI complex that interacts with a conserved region of SNF2. *Mol. Cell. Biol.* **15,** 4240-4248.

Treisman, J. E., Luk, A., Rubin, G. M. and Heberlein, U. (1997). *eyelid* antagonizes *wingless* signaling during *Drosophila* development and has homology to the Bright family of DNA-binding proteins. *Genes Dev.* **11,** 1949-1962.

Tripoulas, N. A., Hersperger, E., LaJeunesse, D. and Shearn, A. (1994). Molecular genetic analysis of the *Drosophila melanogaster* gene *absent, small or homeotic discs 1 (ash1)*. *Genetics* **137,** 1027-1038.

Tripoulas, N. A., LaJeunesse, D., Gildea, J. and Shearn, A. (1996). The *Drosophila ash1* gene product, which is localized at specific sites on polytene chromosomes, contains a SET domain and a PHD finger. *Genetics* **143,** 913-928.

Utley, R. T., Côtè, J., Owen-Hughes, T. and Workman, J. L. (1997). SWI/SNF stimulates the formation of disparate activator-nucleosome complexes but is partially redundant with cooperative binding. *J. Biol. Chem.* **272,** 12642-12649.

Winston, F. and Carlson, M. (1992). Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet.* **8,** 387-391.

Yoshinaga, S. K., Peterson, C. L., Herskowitz, I. and Yamamoto, K. R. (1992). Roles of SWI1, SWI2, and SWI3 proteins for transcriptional enhancement by steroid receptors. *Science* **258,** 1598-1604.