The *Drosophila* BRM complex facilitates global transcription by RNA polymerase II

Jennifer A. Armstrong, Ophelia Papoulas, Gary Daubresse¹, Adam S. Sperling, John T. Lis², Matthew P. Scott¹ and John W. Tamkun³

Department of Molecular, Cell, and Developmental Biology, University of California-Santa Cruz, Santa Cruz, CA 95064, ¹Departments of Developmental Biology and Genetics, Howard Hughes Medical Institute, Beckman Center, Stanford University School of Medicine, Stanford, CA 94305 and ²Department of Molecular Biology and Genetics, Biotechnology Building, Cornell University, Ithaca, NY 14853, USA

³Corresponding author
e-mail: tamkun@biology.ucsc.edu

***Drosophila brahma* (*brm*) encodes the ATPase subunit of a 2 MDa complex that is related to yeast SWI/SNF and other chromatin-remodeling complexes. BRM was identified as a transcriptional activator of Hox genes required for the specification of body segment identities. To clarify the role of the BRM complex in the transcription of other genes, we examined its distribution on larval salivary gland polytene chromosomes. The BRM complex is associated with nearly all transcriptionally active chromatin in a pattern that is generally non-overlapping with that of Polycomb, a repressor of Hox gene transcription. Reduction of BRM function dramatically reduces the association of RNA polymerase II with salivary gland chromosomes. A few genes, such as induced heat shock loci, are not associated with the BRM complex; transcription of these genes is not compromised by loss of BRM function. The distribution of the BRM complex thus correlates with a dependence on BRM for gene activity. These data suggest that the chromatin remodeling activity of the BRM complex plays a general role in facilitating transcription by RNA polymerase II.**

Keywords: Brahma/chromatin remodeling/Polycomb/trithorax group

Introduction

The Homeobox selector (Hox) genes specify the identities of body segments in *Drosophila melanogaster* and other metazoans (Gellon and McGinnis, 1998). The transcription of Hox genes must be regulated precisely, since their misexpression often causes dramatic alterations in cell fates. In *Drosophila*, the initial patterns of Hox gene expression are established early in embryogenesis by transcription factors encoded by segmentation genes. During subsequent development, Polycomb group (PcG) and trithorax group (trxG) proteins assume control of Hox gene transcription (Simon, 1995; Gellon and McGinnis, 1998; Francis and Kingston, 2001). PcG proteins maintain

repressed states of Hox gene transcription while trxG proteins maintain active transcription of Hox genes. Increasing evidence suggests that trxG and PcG proteins regulate transcription by altering chromatin structure (Simon and Tamkun, 2002).

Nucleosomes, the basic units of chromatin, can act as potent repressors of transcription by blocking access of transcription factors and other regulatory proteins to DNA. Two strategies are used to regulate transcription in a chromatin environment: the covalent modification of histone tails and energy-dependent chromatin remodeling (Narlikar *et al.*, 2002). N-terminal histone tails protrude from the surface of the nucleosome where they can mediate interactions with linker DNA (Angelov *et al.*, 2001), adjacent nucleosomes (Luger *et al.*, 1997) or chromatin-associated proteins (Jenuwein and Allis, 2001). The acetylation, methylation or phosphorylation of histone tails can have profound effects on chromatin structure and transcription (Jenuwein and Allis, 2001). Recent studies have revealed connections between several trxG and PcG proteins and histone-modifying enzymes. For example, the trxG protein Trithorax (TRX) physically interacts with the histone acetyltransferase (dCBP), while the PcG proteins Extra sex combs (ESC) and Enhancer of zeste [E(Z)] physically interact with the histone deacetylase Rpd3 (Petruk *et al.*, 2001; Tie *et al.*, 2001). These findings suggest that at least some PcG and trxG proteins may recruit histone-modifying enzymes to regulatory elements in the vicinity of Hox genes, thereby altering local chromatin structure and transcription.

Chromatin remodeling reactions are catalyzed by large protein complexes that use the energy of ATP hydrolysis to alter the structure or positioning of nucleosomes, thereby modulating the access of regulatory proteins and general transcription factors to DNA in the context of chromatin (Sudarsanam and Winston, 2000; Narlikar *et al.*, 2002). A direct connection between trxG proteins and chromatin remodeling was provided by studies of the trxG gene *brahma* (*brm*). *brm* was identified in a screen for extragenic suppressors of *Polycomb* (*Pc*), and was subsequently found to encode the *Drosophila* homolog of the *Saccharomyces cerevisiae* SWI2/SNF2 ATPase (Kennison and Tamkun, 1988; Tamkun *et al.*, 1992). The BRM ATPase is a subunit of a 2 MDa complex that is highly related to yeast SWI/SNF and other chromatin-remodeling complexes, including yeast RSC and human SWI/SNF complexes (Papoulas *et al.*, 1998; Collins *et al.*, 1999; Kal *et al.*, 2000). In addition to BRM, the BRM complex contains the trxG proteins Moira (MOR) and OSA (Collins *et al.*, 1999; Crosby *et al.*, 1999; Vazquez *et al.*, 1999). Thus, at least three trxG proteins function as subunits of a large protein complex that appear to regulate the transcription of Hox genes by catalyzing ATP-dependent alterations in chromatin structure.

A subset of PcG proteins may directly counteract chromatin remodeling catalyzed by the BRM complex. Subunits of the 3 MDa Polycomb repressive complex 1 (PRC1) include the PcG proteins Polycomb (PC), Polyhomeotic (PH) and Posterior sex combs (PSC) (Shao *et al.*, 1999). PRC1 interferes with chromatin remodeling by directly preventing nucleosomal binding by human Brahma-related gene 1 (BRG1) (Francis *et al.*, 2001). The BRG1 ATPase is the catalytic subunit of the human SWI/SNF chromatin-remodeling complexes and is homologous to BRM (Khavari *et al.*, 1993). Like *Drosophila* BRM, BRG1 plays a critical role during development, since mouse embryos homozygous for a *Brg1* null mutation die prior to implantation (Bultman *et al.*, 2000). The antagonism between PRC1 and BRG1 *in vitro* suggests that some PcG proteins preserve the silenced state of a gene by preventing access and subsequent chromatin remodeling by the BRM complex. The role of the BRM complex is not limited to counteracting PcG repression, however, since *Drosophila* *brm* is essential for cell viability and oogenesis (Elfring *et al.*, 1998).

In both yeast and vertebrates, chromatin-remodeling complexes can be targeted to the appropriate promoters through direct interactions with transcriptional activators (Peterson and Logie, 2000; Hassan *et al.*, 2001). Targeting by transcriptional activators may be particularly critical for low-abundance chromatin-remodeling complexes such as yeast SWI/SNF, which is present at 100–200 copies per cell (Cairns *et al.*, 1996). Targeting may be less important for highly abundant chromatin-remodeling complexes, including the *Drosophila* BRM and ISWI complexes. The levels of these complexes approach the mass of the nucleosomes used to package the DNA (Tsukiyama *et al.*, 1995; Elfring *et al.*, 1998), suggesting they may play fairly global roles in modulating chromatin structure or transcription. Consistent with this possibility, recent genetic studies have shown that ISWI modulates higher order chromatin structure over broad chromosomal domains (Deuring *et al.*, 2000).

To clarify the function of the BRM chromatin-remodeling complex, we examined the distribution of the BRM ATPase on larval salivary gland polytene chromosomes. By directly visualizing the genome-wide distribution of a protein on chromosomes, this simple assay often provides unforeseen insights into the protein's function. Here we report the surprising observation that the BRM complex marks the majority of transcriptionally active chromatin. In addition, we present evidence that loss of BRM function dramatically impairs transcription by RNA polymerase II (Pol II). These findings suggest that the BRM complex plays a much more general role in facilitating transcription than previously suspected.

Results

The BRM complex is associated with regions of open chromatin

To visualize directly interactions between the BRM complex and chromatin *in vivo*, we examined the distribution of the BRM protein on larval salivary gland polytene chromosomes. BRM is associated with ~300 sites on the euchromatic arms, including chromosomal puffs

Fig. 1. The BRM protein is associated with chromosome puffs and interbands of salivary gland polytene chromosomes. (A) Distribution of BRM protein on wild-type polytene chromosomes. Arrowhead indicates chromocenter. (B) The top panel shows indirect immunofluorescence using an anti-BRM antibody (green), the second panel is DAPI-stained DNA (blue), the third panel shows the merged images and the bottom panel is a split image. Note that BRM protein is predominantly found in the DAPI interbands. The distal region of chromosome arm 2L is shown.

(Figure 1A). Identical protein distributions are seen using polyclonal antibodies against non-overlapping segments of BRM (data not shown). As shown in Figure 1B, BRM is associated predominantly with interbands: regions of less condensed DNA that therefore stain weakly with DAPI. In contrast, no BRM was detected in the centric heterochromatin of the chromocenter (Figure 1A, arrowhead). The widespread distribution of BRM in interband regions of polytene chromosomes suggests that this protein may play a general role in transcription or other processes, possibly by creating or recognizing regions of open chromatin.

To confirm that the distribution of the BRM protein on polytene chromosomes reflects the distribution of the BRM complex, we compared its distribution with that of another subunit of the BRM complex, BAP55. BAP55 is an actin-related protein that is present at stoichiometric levels in the highly purified BRM complex (Papoulas *et al.*, 1998) and is conserved in SWI/SNF-like complexes in yeast and humans (Boyer and Peterson, 2000). As expected for a member of the BRM complex, BAP55 co-immunoprecipitates with epitope-tagged BRM protein (Figure 2A) and the majority of BAP55 in embryo extracts co-elutes with BRM from a gel filtration column (Figure 2B). The distributions of BRM and BAP55 on polytene chromosomes are highly coincident (Figure 2C), suggesting that the observed BRM protein distribution does indeed reflect the distribution of the BRM complex on salivary gland chromosomes.

Both genetic and biochemical evidence indicate that BRM and PC function antagonistically to each other (Kennison and Tamkun, 1988; Tamkun *et al.*, 1992; Shao *et al.*, 1999; Francis *et al.*, 2001). We therefore compared the chromosomal distributions of the two proteins. As shown in Figure 3, the distributions of BRM and PC on salivary gland polytene chromosomes are very different, with the majority of the ~60 PC sites not overlapping the ~300 BRM sites. This result is consistent with a recent proposal that the PC complex directly blocks the BRM complex from associating with chromatin (Francis *et al.*, 2001).

Fig. 2. BAP55, an actin-related protein, is a subunit of the BRM complex. (A) BAP55 is physically associated with the BRM complex in embryo extracts. Immunoprecipitations were performed using the 12CA5 antibody against the HA tag and protein extracts derived from either OregonR embryos (lanes 1–3) or *P[w⁺, brm-HA-6His]92C; brm²/Df(3L)th102* embryos (lanes 4–6). Western blotting was performed on one-tenth of the total input extract (I) and supernatant (S) and one-fifth of the total pellet (P) using antibodies against BRM, BAP111, BAP55 and RNA Pol II. The HMG-domain protein BAP111, another subunit of the BRM complex, is presented as a positive control (Papoulas *et al.*, 2001). Note that BAP111 and BAP55 are immunoprecipitated with BRM, while Pol II is not (lane 6). Proteins in the pellet lane show slightly reduced mobility relative to start and supernatant due to differences in buffer conditions. (B) Western blot of fractions derived from a Superose 6 gel filtration column loaded with embryo extract and probed with antibodies against BRM, BAP111 and BAP55. Vertical arrows indicate void and elution volumes of molecular weight standards. Note that the majority of BAP55 co-elutes with BRM and BAP111, while some BAP55 appears to be monomeric. (C) Distributions of BRM (green) and BAP55 (red) on wild-type salivary gland polytene chromosomes. Chromosome arm 2L is shown. Note that the patterns of BAP55 and BRM proteins are predominantly overlapping.

The BRM complex is preferentially associated with transcriptionally active chromatin

We next compared the chromosomal distributions of BRM and RNA Pol II. For these experiments, we initially used an antibody directed against subunit IIc of Pol II, which recognizes both the initiating and elongating forms of the enzyme. The relative levels of BRM and Pol II vary at many sites (Figure 4A), but upon close examination it is clear that the BRM complex marks nearly every

transcriptionally active site in the genome (Figure 4B). The C-terminal domain (CTD) of the largest Pol II subunit consists of multiple repeats of the heptad sequence (Tyr-Ser-Pro-Thr-Ser-Pro-Ser). The CTD is phosphorylated on serines at positions 2 and 5 of this repeat during the elongation phase of the transcription cycle. While the CTDs of the initiating and promoter-paused forms of Pol II are hypophosphorylated (Pol IIa) (Weeks *et al.*, 1993; O'Brien *et al.*, 1994; Lis, 1998), Ser5 of the CTD is phosphorylated preferentially near the promoter (Pol IIo^{Ser5}), and Ser2 is phosphorylated on Pol II throughout the transcription unit (Pol IIo^{Ser2}) (Komarnitsky *et al.*, 2000). The distribution of the BRM complex is highly coincident with that of Pol IIo^{Ser2} (Figure 5A), as well as with that of Pol IIa (Figure 5B). Thus, the BRM complex associates with sites of both the initiating or promoter-paused Pol II and mature, elongating Pol II.

The physical association of BRM with almost all active genes in salivary gland nuclei suggests that the BRM complex facilitates transcription, perhaps by increasing the accessibility of chromatin to transcription factors or Pol II. It is also possible, however, that the BRM complex is not required to create regions of active chromatin, but merely recognizes some characteristic feature of actively transcribed genes. For example, the association of the BRM complex with active chromatin could result from a physical association of the BRM complex with Pol II, as has been reported for SWI/SNF in yeast (Wilson *et al.*, 1996). The physical association of yeast SWI/SNF and Pol II has been somewhat controversial, with some groups detecting a physical association while others have not (Cairns *et al.*, 1996). To determine whether the *Drosophila* BRM complex is physically associated with Pol II, we attempted to co-immunoprecipitate Pol II and BRM. We were unable to detect a physical association between BRM and Pol II using very mild conditions (Figure 2A), so we favor a model in which BRM is recruited to active promoters through some other mechanism.

Histone tails can be post-translationally modified by phosphorylation, acetylation and methylation to create distinct chromatin environments, with transcriptionally active chromatin looking very different from inactive chromatin (Jenuwein and Allis, 2001). It is possible that the BRM complex recognizes a histone modification that is enriched in active chromatin. Several protein domains that recognize modified histone tails have recently been identified. For example, the bromodomains of GCN5, TAFII250 and p300/CBP-associated factor (P/CAF) specifically bind acetylated histone tails (Dhalluin *et al.*, 1999; Jacobson *et al.*, 2000; Owen *et al.*, 2000). We therefore wondered whether the bromodomain near the C-terminus of the BRM protein might recruit the BRM complex to active chromatin.

Although the *brm* gene is essential in flies, the bromodomain of BRM is not (Elfring *et al.*, 1998). We were therefore able to examine the distribution of BRM on salivary gland polytene chromosomes from larvae that express only BRM protein lacking the bromodomain (BRM^{ABD}). The chromosomal distribution and level of the BRM protein in wild-type and BRM^{ABD} larvae appears quite similar compared with Pol II (Figure 6), indicating that the BRM bromodomain is not required for the association of the BRM complex with transcriptionally

Fig. 3. The BRM complex is associated with regions that are not bound by PC. Immunofluorescence detection of BRM (green) and PC (red) on wild-type salivary gland polytene chromosomes. Note that the distributions of BRM and PC proteins are dissimilar with few sites of overlap.

Fig. 4. The BRM complex is associated with sites of active Pol II transcription. (A) Immunofluorescence detection of BRM (green) and the 140 kDa IIc subunit of RNA Pol II (red) on wild-type salivary gland polytene chromosomes reveals a high degree of overlap. (B) Magnification of the distal region of 3L illustrates that BRM (green) marks active regions of the chromosome, although BRM protein is not always present at the same levels as Pol II (red). This is shown in the bottom right panel as a split image.

active chromatin. It is therefore not likely that the BRM protein is simply responding to levels of histone acetylation via its bromodomain.

The BRM complex is essential for global transcription

The BRM complex is localized to nearly every active gene in salivary gland nuclei, but is it required for transcription of these genes? *brm* is an essential gene; individuals homozygous for *brm* null alleles die before completing embryogenesis (Elfring *et al.*, 1998). To investigate whether *brm* is required for transcription in salivary gland nuclei, we made use of a GAL4-inducible transgene encoding a dominant-negative form of the BRM protein (BRM^{K804R}). A single amino acid substitution in the highly conserved ATP-binding site of the BRM protein (lysine to arginine at residue 804) eliminates BRM ATPase activity. However, this mutant form of the BRM protein is properly assembled into the BRM complex and therefore antagonizes wild-type *brm* function *in vivo* (Elfring *et al.*, 1998). We used an *hsp70-GAL4* driver under non-heat shock conditions to express BRM^{K804R} in salivary glands, and confirmed expression by western blot analysis (data not shown). For simplicity, these individuals will be referred to as *UASbrm*^{K804R}. Although the mutant salivary glands are reduced in size, this level of expression of BRM^{K804R} does not drastically disrupt the structure of the chromosomes. The DAPI-stained chromosomes are

slightly thinner than control chromosomes derived from control glands expressing LacZ, but otherwise display an overall normal banding pattern (see below). To address whether BRM is necessary for transcription, we examined the distribution of Pol II^{Ser2} on the mutant chromosomes. As one of the predominant forms of elongating Pol II in flies, the presence of Pol II^{Ser2} on polytene chromosomes reflects active transcription (Kaplan *et al.*, 2000; Prelich 2002). Upon BRM^{K804R} expression in the salivary glands, the level of Pol II^{Ser2} on chromosomes is drastically reduced (Figure 7B) as compared with the chromosomes of control glands expressing LacZ (Figure 7A). The BRM complex is not only required for the elongation of Pol II; the levels of initiating and promoter-paused Pol II (Pol IIa) were also reduced on mutant chromosomes (Figure 7D) as compared with control chromosomes (Figure 7C). Thus, a functional BRM complex appears to be required for Pol II association with promoters.

As a specificity control we examined the distribution of other chromatin-binding proteins on salivary gland polytene chromosomes from mutant larvae. The distribution and levels of PC protein appear to be comparable on chromosomes derived from control salivary glands expressing LacZ (Figure 8A, green) and those derived from salivary glands expressing BRM^{K804R} (Figure 8B, green). The levels of PC are occasionally slightly reduced, consistent with thinner chromosomes, but are not disrupted or reduced to the same degree as Pol II^{Ser2}

Fig. 5. The BRM complex co-localizes with both the promoter entry and elongational forms of Pol II. (A) Immunofluorescence detection of BRM (green) and RNA Pol II^{Ser2} (red) on wild-type salivary gland polytene chromosomes reveals that most regions of transcriptional elongation are also bound by the BRM complex. The distal regions of the X and 3L chromosomes are shown. (B) Immunofluorescence detection of BRM (green) and RNA Pol IIa (red) on wild-type salivary gland polytene chromosomes reveals that the BRM complex is present at many sites of transcriptional initiation or promoter pausing. The distal region of the X chromosome is shown.

Fig. 6. The BRM bromodomain is not required for the recruitment of the BRM complex to active chromatin. Immunofluorescence detection of BRM (green) and RNA Pol II (subunit IIc) (red) on salivary gland polytene chromosomes derived from larvae expressing only BRM lacking the bromodomain (BRM^{ABD}). Note that BRM^{ABD} is localized to transcriptionally active regions. The distal region of the X chromosome is shown.

(Figure 8A and B, red). The observation that loss of BRM function does not affect the binding of PC to chromosomes is consistent with genetic studies showing that partial loss of *Pc* function does not suppress *brm* mutant phenotypes (Brizuela *et al.*, 1994). The protein levels and distribution of the ISWI ATPase (a chromatin-remodeling factor related to BRM) are similarly unaffected on polytene chromosomes derived from salivary glands expressing BRM^{K804R} (Figure 8D) as compared with chromosomes derived from control salivary glands (Figure 8C). We therefore conclude that expression of BRM^{K804R} does not non-specifically affect the binding of proteins to chromatin.

Fig. 7. Expression of dominant-negative BRM (BRM^{K804R}) in the salivary gland compromises Pol II transcription on polytene chromosomes. Immunofluorescence using antibodies directed against RNA Pol IIo^{Ser2} (A and B, red) and RNA Pol IIa (C and D, green). Note that expression of dominant-negative BRM (UASbrm^{K804R}) results in a dramatic reduction in the levels of both Pol IIo^{Ser2} (B) and Pol IIa (D) relative to that observed on chromosomes derived from salivary glands expressing LacZ (UASlacZ) (A and C).

Since *brm* is essential, it is possible that the observed reduction in Pol II transcription is a secondary consequence of a general decline in the level or activity of Pol II due to loss of an essential gene. We have conducted two experiments to exclude this possibility. First, we examined the levels of Pol II present in the salivary glands. The ratios of Pol II protein relative to a control protein, α -tubulin, are not reduced in larval salivary glands expressing BRM^{K804R} when compared with glands expressing LacZ (1.8 and 1.5, respectively; Figure 9A). Thus, partial loss of BRM function does not result in a specific decline in the levels of Pol II.

Secondly, to demonstrate that chromosomes derived from salivary glands expressing BRM^{K804R} are still capable of a transcriptional response, we examined the expression of a gene not regulated by BRM. We chose the heat shock genes for these experiments, because genetic studies have suggested that the BRM complex might not be required for transcription from the heat shock promoter (Tamkun *et al.*, 1992). Furthermore, the BRM protein does not localize to the heat shock puffs following heat shock (Figure 9B). Lastly, expression of a dominant-negative form of human BRG1 has no effect on heat shock-induced activation of *hsp70* (de la Serna *et al.*, 2000). Thus, the heat shock genes appeared to be good candidates as controls to determine whether or not salivary glands expressing BRM^{K804R} are competent for transcription. As shown in Figure 9C and D, the heat shock response is intact in glands expressing BRM^{K804R}, since heat shock results in the recruitment of similar levels of Pol IIo^{Ser2} (red) to heat shock loci in polytene chromosomes expressing either LacZ (Figure 9C) or BRM^{K804R} (Figure 9D). These results suggest that loss of BRM function does not result in a non-specific loss of Pol II activity. We therefore conclude that the BRM complex is required for transcription of the majority of Pol II genes.

Fig. 8. Loss of BRM function does not affect the binding of other chromatin-associated proteins to chromosomes. (A and B) Immunofluorescence detection of PC (green) and RNA Pol Ilo^{Ser2} (red). In marked contrast to RNA Pol Ilo^{Ser2} (red), the levels and distribution of PC (green) are comparable on polytene chromosomes derived from *UASlacZ* larvae (A) or *UASbrm*^{K804R} larvae (B). (C–F) The levels and distribution of ISWI (green) are comparable on polytene chromosomes derived from *UASlacZ* larvae (C) or *UASbrm*^{K804R} larvae (D). As an internal control, the chromosomes were simultaneously stained for RNA Pol Ilo^{Ser2} (red). The levels of Pol Ilo^{Ser2} are reduced on chromosomes from *UASbrm*^{K804R} larvae (F) relative to the levels observed on chromosomes from *UASlacZ* (E).

Discussion

In this study we report the unexpected observation that the BRM ATPase marks nearly all transcriptionally active chromatin on polytene chromosomes. Furthermore, partial loss of BRM function drastically reduces global transcription. A few genes, such as induced heat shock loci, are not associated with the BRM complex. Transcription of these genes is not compromised by loss of BRM function. Thus, the distribution of the BRM protein is correlated with a dependence on BRM for gene activity. We therefore conclude that the BRM chromatin-remodeling complex is required for most RNA Pol II transcription in salivary gland nuclei.

Although this study focused on the role of BRM in a polytene tissue, the observations presented here are consistent with genetic studies of diploid tissues. *brm* is required for the expression of a variety of genes in imaginal discs including *Ultrabithorax*, *Antennapedia*, *Sex combs reduced* and *engrailed* (Tamkun *et al.*, 1992; Elfving *et al.*, 1998). Interestingly, the BRM complex has also been implicated in transcriptional repression. The *trxG* gene *osa* (also called *eyelid*) encodes an ARID-domain protein that is present in a subset of BRM complexes (Collins *et al.*, 1999; Vazquez *et al.*, 1999). OSA-containing BRM complexes are required for the repression of Wingless target genes in the embryo and the wing disc (Collins and Treisman, 2000). A dual role for chromatin-remodeling complexes in both the activation and repression of transcription is not unprecedented. For example, the yeast SWI/SNF complex is required for the activation of 126 genes and the repression of 203 genes (Holstege *et al.*, 1998). Close examination of our polytene chromosomes reveals a small number of BRM sites that do not overlap with transcriptionally active chromatin. It is possible that the BRM complex plays a role in transcriptional repression at these sites. However, the majority of the BRM complex is distributed over transcriptionally active chromatin, suggesting that the major function of the

Drosophila BRM complex is that of facilitating gene expression.

How does the BRM complex activate transcription? Our results suggest that the BRM complex is required for a relatively early step in transcription, since partial loss of BRM function results in reduced levels of RNA Pol IIa on salivary gland polytene chromosomes. BRM may be required for the binding of transcriptional activators, assembly of the pre-initiation or promoter-paused complex, and/or recruitment of Pol II. Furthermore, the similar distributions of BRM and elongating Pol II (Pol Ilo^{Ser2}) on salivary gland polytene chromosomes suggest that BRM might also facilitate transcriptional elongation. It is noteworthy that the *hsp70* heat shock genes do not require the BRM complex for their expression. The *hsp70* genes are unusual in that when uninduced the genes exist in a relatively nucleosome-free configuration (Lis, 1998) with a paused RNA Pol II that has produced a short RNA transcript (Rougvié and Lis, 1988). This configuration may not depend upon the BRM complex for transcriptional activity; rather, the open architecture of these promoters may be a consequence of known interactions with the NURF chromatin-remodeling complex and factors residing upstream of heat shock genes (Tsukiyama and Wu, 1995; Xiao *et al.*, 2001).

Our data suggest that the BRM complex recognizes some unique feature of active genes. We investigated whether BRM physically associates with Pol II, as has been reported for yeast SWI/SNF (Wilson *et al.*, 1996). Although the chromosomal distributions of BRM and Pol II proteins are similar, their levels vary dramatically from site to site, suggesting that BRM and Pol II are not present in the same protein complex. In agreement with this, Pol II was not detected in the purified BRM complex (Papoulas *et al.*, 1998) and we were unable to detect a physical association between BRM and Pol II by co-immunoprecipitation. We also investigated whether the association of BRM with active chromatin is dependent on the BRM bromodomain, a domain conserved in many

Fig. 9. Loss of BRM function does not non-specifically affect the level or activity of RNA Pol II. (A) Pol II protein levels are comparable in salivary glands expressing dominant-negative BRM and control glands expressing LacZ. Western blot of 10 salivary glands derived from *UASlacZ* larvae (lane 1) or *UASbrm^{K804R}* larvae (lane 2). The top panel is probed with antibody against the 140 kDa IIC subunit of RNA Pol II and the bottom panel is probed with antibody against α -tubulin. The ratio of protein levels of Pol II to α -tubulin is 1.5 in salivary glands expressing LacZ and 1.8 in salivary glands expressing BRM^{K804R}. (B) Unlike heat shock factor (HSF, red), BRM (green) is not recruited to heat shock loci following heat shock. DNA is stained with DAPI (blue). (C and D) The heat shock response is intact in salivary glands expressing dominant-negative BRM. *UASlacZ* larvae (B) or *UASbrm^{K804R}* larvae (C) were heat shocked and chromosomes were stained with anti-RNA Pol II^{Ser2} (red) and DAPI (blue). Chromosome 3R is shown and the heat shock genes are indicated. Note that levels of Pol II associated with the heat shock genes are comparable in glands expressing LacZ or BRM^{K804R}.

proteins that interact with chromatin (Horn and Peterson, 2001; Marmorstein and Berger, 2001). The bromodomains of GCN5, TAF_{II}250 and P/CAF specifically bind acetylated histone tails (Dhalluin *et al.*, 1999; Jacobson *et al.*, 2000; Owen *et al.*, 2000). Deletion of the BRM bromodomain, however, did not alter the distribution of the BRM protein. We conclude that the BRM protein does not preferentially associate with acetylated chromatin via its bromodomain. Given the importance of post-translational modifications of histone tails in gene expression, it will be interesting to explore other possible connections

between histone modifying enzymes and the BRM complex.

Transcriptional activators can recruit chromatin-remodeling complexes to specific genes (Peterson and Logie, 2000; Hassan *et al.*, 2001). Given that the BRM complex is associated with the majority of active genes, could direct interactions with transcription factors completely account for its distribution? At least one transcription factor, Zeste, is capable of recruiting the BRM complex to specific genes *in vitro* (Kal *et al.*, 2000). Yeast SWI/SNF binds acidic activation domains of GAL4-VP16, Gcn4, Hap4 and Swi5, while human SWI/SNF interacts with c-Myc, MyoD and nuclear hormone receptors, as well as the zinc fingers of EKLF, SP1 and GATA-1 (Hassan *et al.*, 2001). Most promoters and enhancers have binding sites for multiple transcriptional activators. At least one of these activators may have an acidic activation domain, a zinc finger, or another domain that could interact with the BRM complex. It is therefore possible that transcriptional activators might target the BRM chromatin-remodeling complex to its many sites of action.

Our results are also consistent with the proposal that some chromatin-remodeling complexes act as global regulators of chromatin fluidity (Kingston and Narlikar, 1999). In the nucleus, the mass of the BRM complex is equivalent to the mass of the histones (Elfring *et al.*, 1998). Perhaps the essential, abundant BRM complex acts globally to remodel nucleosomes and facilitate transcription. The regulation of this promiscuous complex may hinge upon negative acting factors that function to exclude the BRM complex from inappropriate genes. PC and the PcG proteins are good candidates for these factors. Francis *et al.* (2001) reported that a core PRC1 protein complex (consisting of PC, PSC, PH and dRING1) prevents the human homolog of BRM (BRG1) from binding to chromatin *in vitro*. Since BRG1, PC and PSC are all capable of binding DNA (Quinn *et al.*, 1996; Francis *et al.*, 2001), the authors proposed that this PcG complex might compete with the BRM complex for binding to the linker regions of chromatin. Alternatively, PRC1 might create higher order chromatin structures that are not accessible to the BRM complex. The predominantly non-overlapping distributions of PC and BRM on salivary gland polytene chromosomes are consistent with both of these models. However, these proposed mechanisms are more difficult to reconcile with the heat shock genes, which do not associate with BRM, yet are also not bound by PcG proteins.

Do SWI/SNF complexes act globally to facilitate transcription in other organisms? Humans possess two ATPases highly related to BRM, BRG1 and hBRM, which function as the catalytic subunits of the SWI/SNF-like BAF and PBAF complexes (Narlikar *et al.*, 2002). These complexes have been implicated in the control of a large variety of genes including multiple genes involved in muscle differentiation, Tcf-responsive genes, targets of hormone receptors, human β -globin, α_1 antitrypsin, CSF1 and the class II transactivator gene (Armstrong *et al.*, 1998; Fryer and Archer, 1998; Barker *et al.*, 2001; de la Serna *et al.*, 2001; Liu *et al.*, 2001; Pattenden *et al.*, 2002; Soutoglou and Talianidis, 2002). When BRG1 was expressed in cells lacking both BRG1 and hBRM, 80 genes

were activated and two were repressed (Liu *et al.*, 2001). Yeast also possess two ATPases highly related to BRM: SWI2/SNF2, the catalytic subunit of SWI/SNF; and STH1, the ATPase of the RSC complex (Sudarsanam and Winston, 2000). Whole-genome microarray experiments suggest that the non-essential SWI/SNF complex is required for the correct expression of only 1–6% of genes (Holstege *et al.*, 1998; Sudarsanam *et al.*, 2000). Less is known about the role of the RSC complex, as the genes that encode its subunits are essential (Cairns *et al.*, 1996). However, the distribution of the RSC complex in the yeast genome suggests that it may play a role in the regulation of ~700 targets (Damelin *et al.*, 2002; Ng *et al.*, 2002). RSC was also found to interact with TATA-binding protein (TBP) (Sanders *et al.*, 2002). These results indicate that several of the SWI/SNF-like complexes target a large number of genes and suggest that the role of the *Drosophila* BRM complex in facilitating general transcription may be conserved in other organisms.

Materials and methods

Drosophila stocks and genetic crosses

Flies were raised on cornmeal/molasses/yeast/agar medium containing Tegosept and propionic acid. Unless otherwise indicated, *Drosophila* strains were obtained from the Bloomington Stock Center and are described in FlyBase (<http://www.flybase.org>). To examine the effect of BRM^{K804R} on chromosome structure and function, *Df(1)w67c2 y; P[w⁺ UAS_G-brm^{K804R}-HA-6His]2-2* (Elfring *et al.*, 1998) or *w; P[w⁺mC UAS-lacZ.B]4-2-4B* flies were crossed to *w; P[w⁺mC GAL4-Hsp70.PB]89-2-1* flies, and polytene chromosomes were prepared from the progeny which were either of the genotype: *Df(1)w67c2 y/w; P[w⁺ UAS_G-brm^{K804R}-HA-6His]2-2/P[w⁺mC GAL4-Hsp70.PB]89-2-1* or *w/w; P[w⁺mC UAS-lacZ.B]4-2-4B/P[w⁺mC GAL4-Hsp70.PB]89-2-1*. These larvae are referred to in the text and figures as *UASbrmK804R* and *UASlacZ*, respectively. Flies expressing BRM lacking the bromodomain were as described previously (Elfring *et al.*, 1998) and are of the genotype *w; P[w⁺, brm^{Δ1446–1517}]; brm²/Df(3L)th102*.

Protein biochemistry

Co-immunoprecipitations were performed as described previously (Papoulas *et al.*, 1998) using mouse monoclonal anti-hemagglutinin (HA) antibody 12CA5 (BabCo, Richmond, CA) and affinity-purified rabbit anti-BRM (Elfring *et al.*, 1998), anti-BAP111 (Papoulas *et al.*, 2001) and anti-BAP55 polyclonal antibodies. The rabbit anti-BAP55 polyclonal antibody was directed against amino acids 48–94 fused to glutathione *S*-transferase and affinity purified. Pol II was detected with goat antibody directed against the 140 kDa IIC subunit of Pol II (kind gift from A.Greenleaf; Skantar and Greenleaf, 1995). The mouse anti- α -tubulin antibody was a kind gift from B.Sullivan (Sisson *et al.*, 2000). Gel filtration chromatography was carried out as described previously (Papoulas *et al.*, 1998) using protein extract prepared from 0–12 h *P[w⁺, brm-HA-6His]92C; brm²/Df(3L)th102* embryos.

Analysis of polytene chromosomes

All salivary gland polytene squashes were prepared from third instar larvae maintained at 18°C. Wild-type polytene chromosomes were prepared from OregonR larvae. For heat shock experiments, larvae were heat shocked at 37°C for 20 min and salivary glands were dissected in 0.7% NaCl solution warmed to 37°C to prevent recovery. To compare the levels of various chromatin-associated proteins on polytene chromosomes from larvae expressing either LacZ or BRM^{K804R}, squashes were prepared simultaneously and the images were taken using identical exposures and processed identically. Each result shown is representative of multiple experiments.

Glands were dissected in 0.7% NaCl and fixed for 10 min in 45% acetic acid/1.85% formaldehyde. After squashing, slides were frozen in liquid nitrogen and coverslips were removed. Slides were washed in phosphate-buffered saline (PBS), PBS/1% Triton X-100 and blocked for 30 min in PBS/0.1% Triton X-100/1% BSA (PBS-TB). Squashes were incubated overnight at 4°C in primary antibodies diluted in PBS-TB. Primary

antibodies from different species were incubated with polytenes simultaneously. Affinity-purified rabbit anti-BRM antibodies (directed against the N-terminal amino acids 505–775 or the C-terminal amino acids 1504–1638; Elfring *et al.*, 1998); rat anti-BRM (directed against amino acids 1504–1638); rabbit anti-PC (kind gift from R.Paró; Strutt *et al.*, 1997); goat anti-RNA Pol II (IIC) (kind gift from A.Greenleaf; Skantar and Greenleaf, 1995); rat anti-BAP55 (directed against amino acids 48–94); guinea pig anti-HSF (Andrulis *et al.*, 2000); rabbit anti-ISWI (Tsukiyama *et al.*, 1995); goat anti-Pol IIa (kind gift from A.Greenleaf; Weeks *et al.*, 1993); and mouse IgM anti-Pol IIC^{Ser2} (H5) (kind gift from G.Hartzog; Kim *et al.*, 1997) were all used at 1:100 dilutions. Slides were then washed in PBS, blocked in PBS-TB, and incubated for 1 h at room temperature in the appropriate secondary antibodies (Jackson ImmunoResearch Laboratories) diluted 1:200 in PBS-TB. Slides were washed in PBS and stained with 50 ng/ml DAPI for 4 min, washed in PBS and mounted in Vectashield (Vector Laboratories, Inc.) or in 80% glycerol/PBS/0.1% *n*-propyl gallate. In all double stains, control squashes in which each of the primary antibodies were omitted in turn were done to ensure that no background fluorescence resulted from either antibody (data not shown).

The method used to obtain double stains using BRM and PC rabbit antibodies was adapted from a protocol suggested by Jackson ImmunoResearch Laboratories. Following incubation with PC antibody, biotin-labeled Fab fragments (diluted 1:200) were used as a secondary. Following incubation with TRITC-labeled streptavidin (diluted 1:400), the squashes were incubated with the second primary (anti-BRM), followed by the second secondary (FITC-labeled goat anti-rabbit). Omission of the anti-BRM primary ensured that the anti-PC antibody was completely blocked by the Fab fragments and not available to bind the FITC-labeled goat anti-rabbit. The results obtained from double stains with rabbit anti-BRM and anti-PC are comparable to protein distributions obtained using rat anti-BRM and rabbit anti-PC (data not shown). Polytene images were captured on a Leitz DMIRB inverted photomicroscope equipped with a Leica TCS NT laser confocal imaging system, a Zeiss Inverted Axiovert fluorescent microscope, or a Leica Aristaplan fluorescent microscope. Images were processed using PhotoShop.

Acknowledgements

We thank Kathy Matthews and the Bloomington Stock Center for numerous stocks. We thank Grant Hartzog, Sergio Pimpinelli, Laura Fanti, Maria Berloco, Craig Kaplan, Keith Maggert, Janis Werner and members of our laboratories for helpful discussions and advice; and Davide Corona and Joseph Schulz for critical reading of the manuscript. We thank Bill Sullivan and Lindsay Hinck for the use of microscopes, and Arno Greenleaf, Grant Hartzog, Bill Sullivan, Renato Paró and Matthias Prestel for generously providing antibodies. A.S.S. was supported by a fellowship from the Beckman Scholars' Program. J.A.A. was supported by the Damon Runyon Cancer Research Foundation Fellowship, DRG-1556. M.P.S. is an Investigator of the Howard Hughes Medical Institute. This work was supported by grants from the National Institutes of Health to J.T.L. (GM25232), to M.P.S. (5PO1 CA70404) and to J.W.T. (GM49883).

References

- Andrulis, E.D., Guzmán, E., Döring, P., Werner, J. and Lis, J.T. (2000) High resolution localization of *Drosophila* Spt5 and Spt6 at heat shock genes *in vivo*: roles in promoter proximal pausing and transcription elongation. *Genes Dev.*, **14**, 2635–2649.
- Angelov, D., Vitolo, J.M., Mutskov, V., Dimitrov, S. and Hayes, J.J. (2001) Preferential interaction of the core histone tail domains with linker DNA. *Proc. Natl Acad. Sci. USA*, **98**, 6599–6604.
- Armstrong, J.A., Bieker, J.J. and Emerson, B.M. (1998) A SWI/SNF-related chromatin remodeling complex, E-RC1, is required for tissue-specific transcriptional regulation by EKLF *in vitro*. *Cell*, **95**, 93–104.
- Barker, N., Hurlstone, A., Musisi, H., Miles, A., Bienz, M. and Clevers, H. (2001) The chromatin remodelling factor Brg-1 interacts with β -catenin to promote target gene activation. *EMBO J.*, **20**, 4935–4943.
- Boyer, L.A. and Peterson, C.L. (2000) Actin-related proteins (Arps): conformational switches for chromatin-remodeling machines? *BioEssays*, **22**, 666–672.
- 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.
- Bultman, S. *et al.* (2000) A *Brg1* null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Mol. Cell*, **6**, 1287–1295.
- Cairns, B.R. *et al.* (1996) RSC, an essential, abundant chromatin-remodeling complex. *Cell*, **87**, 1249–1260.
- Collins, R.T. and Treisman, J.E. (2000) Osa-containing Brahma chromatin remodeling complexes are required for the repression of wingless target genes. *Genes Dev.*, **14**, 3140–3152.
- Collins, R.T., Furukawa, T., Tanese, N. and Treisman, J.E. (1999) Osa associates with the Brahma chromatin remodeling complex and promotes the activation of some target genes. *EMBO J.*, **18**, 7029–7040.
- Crosby, M.A., Miller, C., Alon, T., Watson, K.L., Verrijzer, C.P., Goldman-Levi, R. and Zak, N.B. (1999) The trithorax group gene *moira* encodes a Brahma-associated putative chromatin-remodeling factor in *Drosophila melanogaster*. *Mol. Cell Biol.*, **19**, 1159–1170.
- Damelin, M. *et al.* (2002) The genome-wide localization of Rsc9, a component of the RSC chromatin-remodeling complex, changes in response to stress. *Mol. Cell*, **9**, 563–573.
- de la Serna, I.L., Carlson, K.A., Hill, D.A., Guidi, C.J., Stephenson, R.O., Sif, S., Kingston, R.E. and Imbalzano, A.N. (2000) Mammalian SWI-SNF complexes contribute to activation of the *hsp70* gene. *Mol. Cell Biol.*, **20**, 2839–2851.
- de la Serna, I.L., Carlson, K.A. and Imbalzano, A.N. (2001) Mammalian SWI/SNF complexes promote MyoD-mediated muscle differentiation. *Nat. Genet.*, **27**, 187–190.
- Deuring, R. *et al.* (2000) The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure *in vivo*. *Mol. Cell*, **5**, 355–365.
- Dhalluin, C., Carlson, J.E., Zeng, L., He, C., Aggarwal, A.K. and Zhou, M.M. (1999) Structure and ligand of a histone acetyltransferase bromodomain. *Nature*, **399**, 491–496.
- Elfring, L.K. *et al.* (1998) Genetic analysis of *brahma*: the *Drosophila* homolog of the yeast chromatin remodeling factor SWI2/SNF2. *Genetics*, **148**, 251–265.
- Francis, N.J. and Kingston, R.E. (2001) Mechanisms of transcriptional memory. *Nat. Rev. Mol. Cell Biol.*, **2**, 409–421.
- Francis, N.J., Saurin, A.J., Shao, Z. and Kingston, R.E. (2001) Reconstitution of a functional core polycomb repressive complex. *Mol. Cell*, **8**, 545–556.
- Fryer, C.J. and Archer, T.K. (1998) Chromatin remodelling by the glucocorticoid receptor requires the BRG1 complex. *Nature*, **393**, 88–91.
- Gellon, G. and McGinnis, W. (1998) Shaping animal body plans in development and evolution by modulation of Hox expression patterns. *BioEssays*, **20**, 116–125.
- Hassan, A.H., Neely, K.E., Vignali, M., Reese, J.C. and Workman, J.L. (2001) Promoter targeting of chromatin-modifying complexes. *Front. Biosci.*, **6**, D1054–D1064.
- Holstege, F.C., Jennings, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.R., Lander, E.S. and Young, R.A. (1998) Dissecting the regulatory circuitry of a eukaryotic genome. *Cell*, **95**, 717–728.
- Horn, P.J. and Peterson, C.L. (2001) The bromodomain: a regulator of ATP-dependent chromatin remodeling? *Front. Biosci.*, **6**, D1019–D1023.
- Jacobson, R.H., Ladurner, A.G., King, D.S. and Tjian, R. (2000) Structure and function of a human TAF_{II}250 double bromodomain module. *Science*, **288**, 1422–1425.
- Jenuwein, T. and Allis, C.D. (2001) Translating the histone code. *Science*, **293**, 1074–1080.
- Kal, A.J., Mahmoudi, T., Zak, N.B. and Verrijzer, C.P. (2000) The *Drosophila* Brahma complex is an essential coactivator for the trithorax group protein Zeste. *Genes Dev.*, **14**, 1058–1071.
- Kaplan, C.D., Morris, J.R., Wu, C. and Winston, F. (2000) Spt5 and Spt6 are associated with active transcription and have characteristics of general elongation factors in *D.melanogaster*. *Genes Dev.*, **14**, 2623–2634.
- 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.
- Khavari, P.A., Peterson, C.L., Tamkun, J.W., Mendel, D.B. and Crabtree, G.R. (1993) BRG1 contains a conserved domain of the SWI2/SNF2 family necessary for normal mitotic growth and transcription. *Nature*, **366**, 170–174.
- Kim, E., Du, L., Bregman, D.B. and Warren, S.L. (1997) Splicing factors associate with hyperphosphorylated RNA polymerase II in the absence of pre-mRNA. *J. Cell Biol.*, **136**, 19–28.
- Kingston, R.E. and Narlikar, G.J. (1999) ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. *Genes Dev.*, **13**, 2339–2352.
- Komarnitsky, P., Cho, E.J. and Buratowski, S. (2000) Different phosphorylated forms of RNA polymerase II and associated mRNA processing factors during transcription. *Genes Dev.*, **14**, 2452–2460.
- Lis, J. (1998) Promoter-associated pausing in promoter architecture and postinitiation transcriptional regulation. *Cold Spring Harb. Symp. Quant. Biol.*, **63**, 347–356.
- Liu, R., Liu, H., Chen, X., Kirby, M., Brown, P.O. and Zhao, K. (2001) Regulation of CSF1 promoter by the SWI/SNF-like BAF complex. *Cell*, **106**, 309–318.
- Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. and Richmond, T.J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*, **389**, 251–260.
- Marmorstein, R. and Berger, S.L. (2001) Structure and function of bromodomains in chromatin-regulating complexes. *Gene*, **272**, 1–9.
- Narlikar, G.J., Fan, H.-Y. and Kingston, R.E. (2002) Cooperation between complexes that regulate chromatin structure and transcription. *Cell*, **108**, 475–487.
- Ng, H.H., Robert, F., Young, R.A. and Struhl, K. (2002) Genome-wide location and regulated recruitment of the RSC nucleosome-remodeling complex. *Genes Dev.*, **16**, 806–819.
- O'Brien, T., Hardin, S., Greenleaf, A. and Lis, J.T. (1994) Phosphorylation of RNA polymerase II C-terminal domain and transcriptional elongation. *Nature*, **370**, 75–77.
- Owen, D.J., Ornaghi, P., Yang, J.C., Lowe, N., Evans, P.R., Ballario, P., Neuhaus, D., Filetici, P. and Travers, A.A. (2000) The structural basis for the recognition of acetylated histone H4 by the bromodomain of histone acetyltransferase gcn5p. *EMBO J.*, **19**, 6141–6149.
- Pattenden, S.G., Klose, R., Karaskov, E. and Bremner, R. (2002) Interferon-γ-induced chromatin remodeling at the CIITA locus is BRG1 dependent. *EMBO J.*, **21**, 1978–1986.
- 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*, **125**, 3955–3966.
- Papoulas, O., Daubresse, G., Armstrong, J.A., Jin, J., Scott, M.P. and Tamkun, J.W. (2001) The HMG-domain protein BAP111 is important for the function of the BRM chromatin-remodeling complex *in vivo*. *Proc. Natl Acad. Sci. USA*, **98**, 5728–5733.
- Peterson, C.L. and Logie, C. (2000) Recruitment of chromatin remodeling machines. *J. Cell Biochem.*, **78**, 179–185.
- Petruk, S., Sedkov, Y., Smith, S., Tillib, S., Kraevski, V., Nakamura, T., Canaani, E., Croce, C.M. and Mazo, A. (2001) Trithorax and dCBP acting in a complex to maintain expression of a homeotic gene. *Science*, **294**, 1331–1334.
- Prelich, G. (2002) The RNA polymerase II CTD kinases: emerging clues to their function. *Eukaryot. Cell*, **1**, 153–162.
- Quinn, J., Fyrberg, 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.
- Rougvie, A.E. and Lis, J.T. (1988) The RNA polymerase II molecule at the 5' end of the uninduced *hsp70* gene of *D.melanogaster* is transcriptionally engaged. *Cell*, **54**, 795–804.
- Sanders, S.L., Jennings, J., Canutescu, A., Link, A.J. and Weil, P.A. (2002) Proteomics of the eukaryotic transcription machinery: identification of proteins associated with components of yeast TF_{II}D by multidimensional mass spectrometry. *Mol. Cell Biol.*, **22**, 4723–4738.
- Shao, Z., Raible, F., Mollaaghababa, R., Guyon, J.R., Wu, C.T., Bender, W. and Kingston, R.E. (1999) Stabilization of chromatin structure by PRC1, a Polycomb complex. *Cell*, **98**, 37–46.
- Simon, J. (1995) Locking in stable states of gene expression: transcriptional control during *Drosophila* development. *Curr. Biol.*, **7**, 373–385.
- Simon, J. and Tamkun, J.W. (2002) Programming off and on states in chromatin: mechanisms of Polycomb and trithorax group complexes. *Curr. Opin. Genet. Dev.*, **12**, 210–218.
- Sisson, J.C., Field, C., Ventura, R., Royou, A. and Sullivan, W. (2000) Lava lamp, a novel peripheral golgi protein, is required for *Drosophila melanogaster* cellularization. *J. Cell Biol.*, **151**, 905–918.
- Skantar, A.M. and Greenleaf, A.L. (1995) Identifying a transcription factor interaction site on RNA polymerase II. *Gene Expr.*, **5**, 49–69.
- Soutoglou, E. and Talianidis, I. (2002) Coordination of PIC assembly and

- chromatin remodeling during differentiation-induced gene activation. *Science*, **295**, 1901–1904.
- Strutt,H., Cavalli,G. and Paro,R. (1997) Co-localization of Polycomb protein and GAGA factor on regulatory elements responsible for the maintenance of homeotic gene expression. *EMBO J.*, **16**, 3621–3632.
- Sudarsanam,P. and Winston,F. (2000) The Swi/Snf family nucleosome-remodeling complexes and transcriptional control. *Trends Genet.*, **16**, 345–351.
- Sudarsanam,P., Iyer,V.R., Brown,P.O. and Winston,F. (2000) Whole-genome expression analysis of *snf1swi* mutants of *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **97**, 3364–3369.
- Tamkun,J.W., Deuring,R., Scott,M.P., Kissinger,M., Pattatucci,A.M., Kaufman,T.C. and Kennison,J.A. (1992) *brhma*: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell*, **68**, 561–572.
- Tie,F., Furuyama,T., Prasad-Sinha,J., Jane,E. and Harte,P.J. (2001) The *Drosophila* Polycomb group proteins ESC and E(Z) are present in a complex containing the histone-binding protein p55 and the histone deacetylase RPD3. *Development*, **128**, 275–286.
- Tsukiyama,T. and Wu,C. (1995) Purification and properties of an ATP-dependent nucleosome remodeling factor. *Cell*, **83**, 1011–1020.
- Tsukiyama,T., Daniel,C., Tamkun,J. and Wu,C. (1995) ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. *Cell*, **83**, 1021–1026.
- Vazquez,M., Moore,L. and Kennison,J.A. (1999) The trithorax group gene *osa* encodes an ARID-domain protein that genetically interacts with the Brahma chromatin-remodeling factor to regulate transcription. *Development*, **126**, 733–742.
- Weeks,J.R., Hardin,S.E., Shen,J., Lee,J.M. and Greenleaf,A.L. (1993) Locus-specific variation in phosphorylation state of RNA polymerase II *in vivo*: correlations with gene activity and transcript processing. *Genes Dev.*, **7**, 2329–2344.
- Wilson,C.J., Chao,D.M., Imbalzano,A.N., Schnitzler,G.R., Kingston,R.E. and Young,R.A. (1996) RNA polymerase II holoenzyme contains SWI/SNF regulators involved in chromatin remodeling. *Cell*, **84**, 235–244.
- Xiao,H., Sandaltzopoulos,R., Wang,H.M., Hamiche,A., Ranallo,R., Lee,K.M., Fu,D. and Wu,C. (2001) Dual functions of largest NURF subunit NURF301 in nucleosome sliding and transcription factor interactions. *Mol. Cell*, **8**, 531–543.

*Received March 26, 2002; revised July 8, 2002;
accepted August 13, 2002*