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Native and Recombinant Polycomb Group Complexes Establish a Selective Block to Template Accessibility To Repress Transcription In Vitro

Ian F. G. King, Nicole J. Francis, and Robert E. Kingston*

Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114, and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115

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Polycomb group (PcG) proteins are responsible for stable repression of homeotic gene expression during *Drosophila melanogaster* development. They are thought to stabilize chromatin structure to prevent transcription, though how they do this is unknown. We have established an in vitro system in which the PcG complex PRC1 and a recombinant PRC1 core complex (PCC) containing only PcG proteins are able to repress transcription by both RNA polymerase II and by T7 RNA polymerase. We find that assembly of the template into nucleosomes enhances repression by PRC1 and PCC. The subunit Psc is able to inhibit transcription on its own. PRC1- and PCC-repressed templates remain accessible to Gal4-VP16 binding, and incubation of the template with HeLa nuclear extract before the addition of PCC eliminates PCC repression. These results suggest that PcG proteins do not merely prohibit all transcription machinery from binding the template but instead likely inhibit specific steps in the transcription reaction.

For a complex multicellular organism to develop properly, it must faithfully execute a complex pattern of gene expression throughout the course of development. Early in *Drosophila melanogaster* development, a body pattern is laid down by homeotic genes, specifying the identity that each body segment will take in the fully developed organism. The factors that establish this pattern are expressed only transiently, but it is essential that the pattern be maintained throughout development. A separate set of factors encoded by the Polycomb group (PcG) and trithorax group (trxG) genes are responsible for maintaining homeotic gene expression patterns.

The PcG genes are responsible for repressing gene expression in appropriate areas during the development of the *Drosophila* embryo (18, 26, 52). In PcG mutant flies homeotic gene expression patterns are established normally, but as development progresses they are not properly maintained, and homeotic genes are expressed ectopically (30, 51, 55, 58). PcG proteins appear to repress gene expression at the transcriptional level: when PcG proteins are artificially targeted to transfected reporter constructs in HeLa cells (11) or to reporter transgenes in *Drosophila* (34, 44), they repress the expression of those reporters. In both HeLa cells and *Drosophila* mRNA levels of PcG-repressed genes are sharply reduced (11, 55, 58).

Genetic evidence suggests that PcG proteins may repress gene activity through a mechanism that involves chromatin structure. Screens for suppressors of PcG mutations have identified a class of genes termed the trithorax group, which are essential for maintaining the activation of homeotic gene expression during development (27, 28), and among this class of genes are a number known to be involved in modulating chromatin structure. Most notably, trxG proteins Brahma, Moira, and Osa are found in a complex in *Drosophila* that is homologous to the Swi/Snf family of chromatin-remodeling complexes (16, 24, 39). The trxG protein Kismet also shares homology to ATP-dependent chromatin-remodeling proteins (14). Additionally, the Trithorax protein is found in a complex with the histone acetyltransferase CBP (4, 42). The system used to keep homeotic genes active involves chromatin structure and genetically antagonizes the function of PcG genes.

PcG proteins themselves also contain domains that suggest a role in chromatin structure. The Polycomb protein itself binds nucleosome cores through a C-terminal domain (8). Polycomb also contains a chromodomain, a motif also found in a number of other proteins involved in modifying chromatin structure (21, 23, 41, 56, 59). The chromodomain of HP1 specifically recognizes histone H3 tails methylated at lysine 9 (3, 29), while the chromodomains of MOF (1) and dMi2 (7) have been shown previously to bind RNA and DNA, respectively. Additionally, the Enhancer of zeste [E(z)] PcG protein contains a SET domain, which is a histone methyltransferase domain in the Su(var)3-9 protein (13, 45), and a histone tail-binding domain in Trithorax (25). It thus seems likely that the PcG complexes function via interactions with chromatin.

Accordingly, in one model for PcG gene repression PcG proteins create a chromatin environment or conformation that prevents all or part of the transcription machinery from accessing the DNA. This hypothesis has been tested in vivo by determining the accessibility of PcG-repressed chromatin to various probes. Transcription of transgenes by T7 RNA polymerase is inhibited when they are inserted into some parts of the PcG-repressed bithorax homeotic gene complex, but not others (17). Similarly, binding of the Gal4 activator and Pc to a transgene containing a PcG targeting element appears to be mutually exclusive, though it has been examined only at the

* Corresponding author. Mailing address: Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114. Phone: (617) 726-5990. Fax: (617) 726-5949. E-mail: Kingston@frodo.mgh.harvard.edu.

level of immunofluorescence (62). PcG repression also seems to reduce accessibility to Flp recombinase (17) and dam DNA methylase (6). Restriction enzymes, however, are able to fully access PcG-repressed sequences in vivo (48). Like these probes, all or part of the transcription machinery may be excluded from chromatin by PcG proteins.

Alternatively, PcG proteins might also interact directly with components of the transcription machinery to repress their activity. For example, PcG proteins interact with TATA-binding protein (TBP)-associated factor (TAF) proteins of the TFIID complex (47), and general transcription factors TFIIB, TFIIF, and TBP are present on PcG-repressed genes in cultured cells (9). However, the functional significance of these interactions is not clear. It is also possible that PcG proteins use a combination of both types of mechanisms to repress gene expression.

To understand how PcG proteins repress gene activity, we established an in vitro system to study transcriptional repression by PcG complexes. We tested whether a PcG complex purified from *Drosophila* embryos, Polycomb repressive complex 1 (PRC1) (49), was able to inhibit transcription in vitro. PRC1 contains stoichiometric amounts of four PcG proteins, along with a number of other subunits (47). These include TAFs, also found in the general transcription factor TFIID. We also tested a recombinant PRC1 core complex (PCC) (19) composed of only the four PcG subunits of PRC1.

In this system, PRC1 and PCC both inhibit transcription by RNA polymerase II (Pol II) and by T7 RNA polymerase. Interestingly, we find that both PRC1 and PCC inhibit transcription at lower concentrations when the template is assembled into nucleosomes than when it is not. Order-of-addition experiments also suggest that PCC inhibits a step or steps in transcription after activator binding. Further, PcG complexes do not simply render the template inaccessible to all factors—binding of the activator Gal4-VP16 is not blocked by PRC1 or PCC. PcG complexes may selectively limit access of DNA binding factors to the template, repressing transcription by excluding parts of the transcription machinery.

MATERIALS AND METHODS

Insertion of T7 RNA polymerase start site into 5S array. A PCR strategy was used to replace the sequence from +55 to +81 after the E4 start site of the pG5E4 plasmid (57) with the sequence 5′TAATACGACTCACTATAGGGAG ATCT3′, which contains a start site for T7 RNA polymerase and a *Bgl*II site.

Protein purification. Gal4-VP16 was prepared as described previously (10). PRC1 was prepared as described previously (49). Approximate molar concentrations were determined by comparison to recombinant PCC preparations by Western blotting. PCC was prepared as described previously (19). Human Swi/Snf (hSwi/Snf) was prepared as described previously (51). HeLa nuclear extract was prepared as described previously (15). HeLa core histones were prepared as described previously (60). Psc, dRING1, Ph, and Pc were prepared as described previously (19), except that all were purified from nuclear extracts, and all were purified by using only an M2 immunoaffinity column, omitting other purification steps.

Assembly of 5S nucleosomal arrays. Following digestion of pG5E4 with *Cla*I, Asp718, and *Dde*I, 5S array DNA fragments for transcription assays were purified on a Sephacryl S-1000 column in Tris-EDTA. Fractions containing the 5S array fragment were pooled, ethanol precipitated, and resuspended in Tris-EDTA. Nucleosomal templates were assembled by salt dialysis as described previously (12, 50). Assemblies were analyzed by *Eco*RI digestion (12) and agarose gel mobility shift (12, 54).

In vitro transcription. Transcription reactions were carried out essentially as described previously (36, 54). Twenty nanograms (15.2 fmol) of assembled 5S nucleosomal array template or unassembled naked 5S array DNA was incubated with PcG complexes (for PRC1, between 2 and 60 fmol; for PCC, between 11 and 140 fmol) for 15 min at 30°C in 60% BC100 (12 mM HEPES [pH 7.9], 60 mM KCl, 12% glycerol, 0.12 mM EDTA) supplemented with $MgCl_2$ to 2 mM and bovine serum albumin (BSA) to 0.5 mg/ml. Gal4-VP16 was added to a concentration determined empirically to optimize transcription, approximately a sixfold molar excess over Gal4 sites. Gal4-VP16 was bound for 15 min at room temperature in 60% BC100, with 0.2 to 0.7 μM $ZnCl_2$ and 2 mM $MgCl_2$. For binding and remodeling with hSwi/Snf, 200 to 300 ng of affinity-purified Swi/Snf was added, along with ATP to 1 mM and $MgCl_2$ to 2 mM. hSwi/Snf was incubated with the template for 30 min at 30°C. Fifty to 100 μg of HeLa nuclear extract was added to the reaction mixture, along with HEPES (pH 7.6) to 22 mM, KCl to 60 mM, $MgCl_2$ to 7 mM, polyvinyl alcohol to 2%, 300 ng of poly(dI-dC) (Roche), and 20 U of RNasin (Promega). Templates were incubated with HeLa nuclear extract for 20 min at room temperature. In order-of-addition experiments (Fig. 5), poly(dI-dC) was omitted from the reaction mixture and $MgCl_2$ was added to 2 rather than 7 mM for incubation with HeLa nuclear extract and transcription.

To transcribe the template, nucleoside triphosphates (NTPs) were added to 0.6 mM, and reaction mixtures were incubated at 30°C for 30 min. Final reaction mixture volumes were between 45 and 60 μl. Reactions were stopped with 75 μl of stop solution (6.6 mM EDTA, 66 mM sodium acetate, 3% sodium dodecyl sulfate, 0.66 mg of tRNA/ml), and reaction mixtures were extracted twice with phenol-chloroform-isoamyl alcohol (pH 4.7; 125:24:1; Sigma) and ethanol precipitated. Primer extension was performed as described previously (53, 54) with an oligonucleotide primer corresponding to +86 to +110 after the E4 start site (36) and Moloney murine leukemia virus reverse transcriptase (Gibco). Primer extension products were visualized on 8% denaturing polyacrylamide gels and quantitated with a phosphorimager (Molecular Dynamics).

T7 RNA polymerase transcription. Twenty nanograms of naked DNA or nucleosomal array templates was incubated with PRC1 or PCC as described for Pol II transcription. An equal volume of a 1× T7 reaction buffer (New England Biolabs [NEB]) with 20 U of RNasin, 1 mM (each) NTP, 200 ng of BSA/μl, and 0.1 U of T7 RNA polymerase (NEB) was added to the PRC1 or PCC reaction mixture, and the template was transcribed for 10 min at 37°C. The final reaction mixture volume was 20 μl. The reactions were stopped as for Pol II transcription, and primer extension was performed as for Pol II transcription. Products were visualized on 20% denaturing polyacrylamide gels and quantitated with a phosphorimager.

For reactions in which templates were challenged with *Hha*I, 5S array template was incubated for 10 min at 37°C in 1× NEB buffer 4 plus 1 mg of BSA/ml with or without 20 U of *Hha*I (NEB). From these digestions, aliquots containing 20 ng of template were added to T7 transcription reaction mixtures with PRC1 as described above. Primer extension was performed with a primer to +117 to +143 after the E4 start site, and products were visualized on 8% denaturing polyacrylamide gels and quantitated with a phosphorimager.

DNase I footprinting. 5S array templates were prepared as described above but were end labeled by Klenow (NEB) treatment. Reactions were performed as for transcription, with a mixture of labeled and unlabeled templates totaling 40 ng per reaction mixture. After Gal4-VP16 binding, the reaction mixtures were split, with one 20-ng portion being analyzed for transcription as described above and the other being further divided and digested with different concentrations of DNase I. For digestions, DNase I (Promega) was diluted in buffer containing 10% glycerol, 10 mM NaCl, 40 mM Tris (pH 8), and 20 mM $CaCl_2$. Two microliters was added to each reaction mixture and incubated for exactly 2 min at room temperature. Reactions were stopped with 4 μl of stop solution (100 mM EDTA, 50 mM Tris [pH 8], 1% sodium dodecyl sulfate, 25% glycerol) plus 20 μg of proteinase K (Sigma), and reaction mixtures were digested for 30 min at 55°C. Samples were loaded on a 1.5% agarose gel (26 by 12.5 cm, 200-ml volume) in Tris-borate-EDTA and run at 60 V overnight.

RESULTS

PcG complexes repress transcription by RNA Pol II in vitro. Because PcG proteins are thought to act through a mechanism involving chromatin structure, we first asked whether the PRC1 complex could repress transcription of in vitro-assembled nucleosomal templates. We used a previously characterized chromatin template (22, 36) that allowed us to examine activator-dependent transcription on chromatin. The template contains five Gal4 binding sites upstream of the adenovirus E4 promoter and contains repeats of a 5S positioning sequence

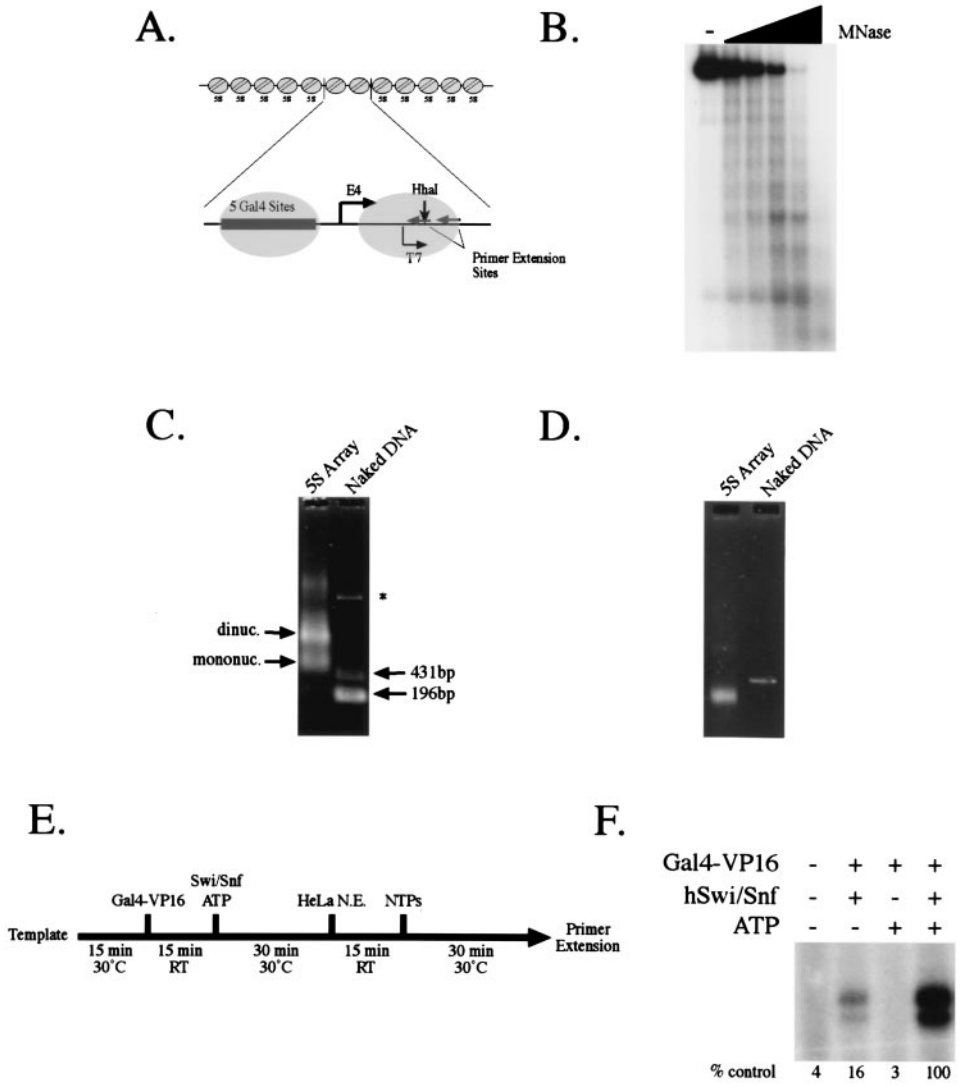

FIG. 1. (A) 5S array transcription template. The template is made up of repeats of a 5S nucleosome positioning sequence flanking a region of 390 bp. This region contains five Gal4 binding sites, the adenovirus E4 promoter, and a start site for T7 RNA polymerase. The relative locations of primers used to visualize RNA transcription products by primer extension are indicated. (B) Micrococcal nuclease (MNase) analysis of 5S nucleosomal array template. (C) *Eco*RI digest of 5S array template. Assembled 5S arrays were digested with *Eco*RI, which cuts between each 5S repeat, reducing the template to 10 196-bp 5S repeat fragments plus one fragment of 431 bp containing the E4 promoter and Gal4 sites. Saturation of assembly is assessed by the proportion of nonnucleosomal mononucleosome-sized fragments. The asterisk indicates the uncut template. (D) Mobility shift analysis. Assembled 5S array templates were compared to naked DNA for a mobility shift indicative of template compaction due to the addition of nucleosomes. (E) Schematic of transcription reaction protocol. RT, room temperature; N.E., nuclear extract. (F) Transcription of the 5S array template is stimulated by hSwi/Snf. Reaction mixtures use 20 ng (~15 fmol) of 5S nucleosomal array, with and without hSwi/Snf and ATP. hSwi/Snf was added to a molar ratio of approximately one per two nucleosomes. Quantitation is relative to +Gal4-VP16–+Swi/Snf lane.

flanking the Gal4 sites and E4 promoter (Fig. 1A). This template can be assembled into a regularly spaced linear nucleosomal array by salt dialysis. We have also inserted a promoter for T7 RNA polymerase downstream of the E4 promoter, allowing us to measure effects of PRC1 complexes on T7 transcription.

We characterized the template by standard techniques to verify that salt dialysis had properly assembled a nucleosomal array (12). Digestion with micrococcal nuclease produced a ladder characteristic of a regularly spaced nucleosomal array (Fig. 1B). We determined the degree of saturation of assembly by digesting the template with *Eco*RI. Sites for *Eco*RI are found in the spacer regions between each 5S positioning sequence of the array template, and so a complete digestion of the template produces mononucleosome- and dinucleosome-sized products. Nonnucleosomal 5S repeats will be visible as an unshifted band after electrophoresis on an agarose gel (gel shift). We used array templates assembled to near 100% saturation, typically more than 90% saturated (Fig. 1C). When a 5S array template is assembled into nucleosomes, it also becomes more compacted than the naked DNA, which alters its mobility on an agarose gel. As has been observed for similar

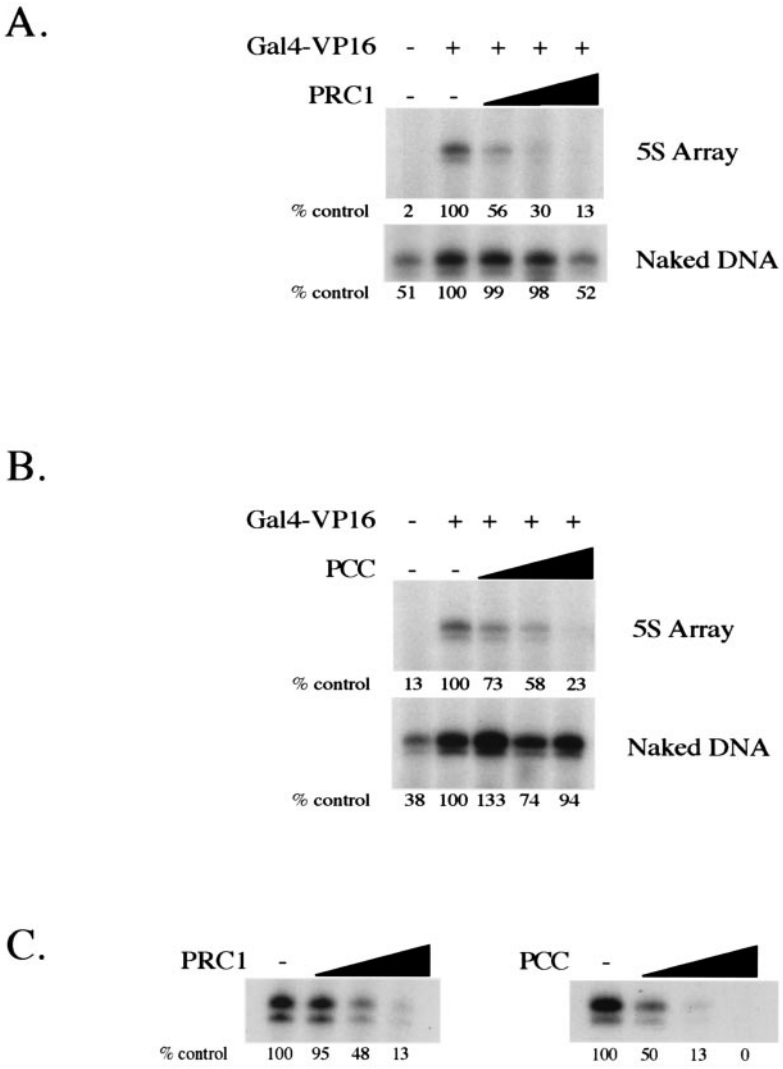

FIG. 2. PRC1 and PCC repress transcription on naked and chromatinized templates. PRC1 (A) and PCC (B) were titrated into transcription reaction mixtures by the same protocol as shown in Fig. 1, including a remodeling step with Swi/Snf and ATP. In both cases, 20 ng of chromatinized 5S template or 20 ng of naked 5S template DNA was used. Reaction mixtures contained 10, 20, and 40 fmol of PRC1 (approximately 0.7:1, 1.3:1, and 2.7:1 molar ratio to template, respectively) and 11, 23, and 47 fmol of PCC (approximately 0.7:1, 1.5:1, and 3:1 molar ratio to template, respectively). Exposure time for naked DNA and chromatin reactions was equal for all reactions shown here. Typically, though, transcription on chromatin is less efficient than transcription on naked DNA. Quantitation is relative to −PcG lane for each. (C) PRC1 and PCC were titrated into transcription reaction mixtures on naked DNA templates at higher ratios to template. Reactions contained 7.5, 15, and 30 fmol of PRC1 and 5 ng of naked DNA template (approximately 2:1, 4:1, and 8:1 molar ratio to template, respectively) or 47, 94, and 188 fmol of PCC and 20 ng of naked DNA template (approximately 3:1, 6:1, and 12:1 molar ratio to template, respectively). Quantitation is relative to −PcG lane for each. All experiments were performed at least three times, and results of representative experiments are shown.

templates, the 5S array template had greater mobility than did naked DNA, indicating that it had been properly assembled into nucleosomes (Fig. 1D).

We sought to establish a system in which hSWI/SNF would affect transcription, since genetic and biochemical studies suggest that PRC1 might directly counteract SWI/SNF function (19, 27, 28, 49). Transcription was carried out by a multistep protocol, sequentially adding the Gal4-VP16 activator, Swi/Snf complex, and HeLa nuclear extract (Fig. 1E). Previously, it was reported that the yeast Swi/Snf complex could activate transcription of this nucleosomal template in conjunction with Gal4-VP16 and that a large part of this activation was ATP dependent (36). In order to verify that this system retained its previously reported characteristics when hSwi/Snf complex was used, we tested the effect of the activator Gal4-VP16 and hSwi/Snf on transcription of the 5S array template. As has been previously reported, transcription is stimulated by Gal4-VP16 (Fig. 2A and B), and when the template is nucleosomal, transcription is stimulated by the addition of the hSwi/Snf complex (Fig. 1F).

Having established a system for activated Pol II transcription of a chromatin template, we tested the effect of PRC1 on transcription in this system. When PRC1 was incubated with the 5S array template before an in vitro transcription reaction,

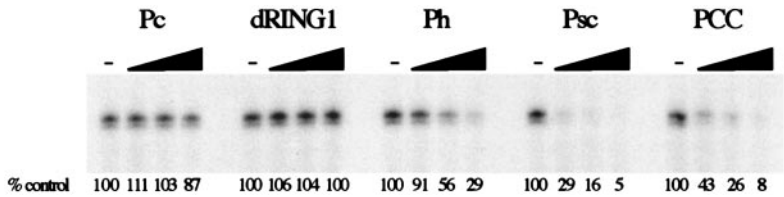

FIG. 3. Analysis of individual PCC subunits in transcription. The subunits that make up PCC were purified individually and added to in vitro transcription reaction mixtures. Titrations for each subunit contained 10, 20, and 40 fmol of total protein and 5 ng of 5S nucleosomal array template (molar ratio of approximately 2.5, 5, and 10 per template, respectively). Also shown is a titration of PCC at these concentrations.

it was able to efficiently repress transcription of the nucleosomal template (Fig. 2A). PRC1 was able to inhibit transcription at low molar ratios to template (approximately one PRC1 per template).

To determine whether repression by PRC1 was an activity of its PcG subunits, we tested whether a highly pure recombinant PCC could also inhibit transcription in vitro. In addition to PcG proteins, PRC1 contains many other subunits that are involved in transcription and might regulate transcriptional repression by PRC1 (47). Most notably, PRC1 contains TAFs, which are also found in the general transcription factor TFIID, the yeast SAGA histone acetyltransferase complex (20), and the human PCAF complex (37). PCC is a stable complex of only the core PcG subunits of the PRC1 complex—Polycomb, Polyhomeotic, Posterior Sex Combs, and dRING1. PCC was also able to inhibit transcription on a nucleosomal template (Fig. 2B). Like PRC1, PCC inhibited transcription at a low ratio to template (approximately five PCCs per template). These experiments demonstrate that the core complex alone can repress transcription, though a higher molar ratio to template than that for PRC1 is needed.

Numerous studies have suggested that PcG complexes in general, and PRC1 in particular, act on chromatin to create a repressed state (18, 43, 52). Thus, PRC1 might collaborate with nucleosome structure to efficiently repress transcription. To determine whether PRC1 required a nucleosomal template to repress transcription, we compared transcription in the presence of either PRC1 or PCC on nucleosomal templates to transcription on naked DNA templates (Fig. 2). Both PRC1 and PCC repress transcription from naked DNA, but repression is less efficient than transcription on a nucleosomal template. Typically, a four- to sixfold-greater concentration of PRC1 or PCC was needed to see similar repression of a naked template. Thus, both PRC1 and PCC are able to repress transcription on naked templates. However, assembling the templates into nucleosomes increases the efficiency of repression.

To determine if any of the four PcG proteins that make up PCC are active on their own, we tested each of the subunits individually for the ability to repress transcription (Fig. 3). Purified Pc, dRING1, Ph, and Psc were incubated with a nucleosomal array template before an in vitro transcription reaction at ratios to template for which PCC is active. The Psc protein alone was able to inhibit transcription approximately as efficiently as PCC. Ph also was able to inhibit transcription, but to a lesser degree. Pc and dRING1 had no activity at these concentrations. We conclude that Psc efficiently inhibits transcription outside the context of PCC and that Ph can also inhibit transcription on its own, but to a lesser extent.

PRC1 and PCC repress transcription by T7 RNA polymerase. PRC1 and PCC could block any number of steps in the transcription reaction. Pol II transcription is a highly complex process requiring many protein factors and many steps (35, 46, 61): binding of activators; recruitment of general transcription factors, RNA Pol II, and other cofactors into a preinitiation complex; initiation of Pol II; and elongation through the template. To determine whether PcG complexes repress transcription by interfering with a reaction step unique to RNA Pol II transcription, we tested the effect of PcG complexes on transcription by T7 RNA polymerase. Transcription by T7 RNA polymerase is a relatively simple process: T7 polymerase is a single-subunit polymerase that directly recognizes a specific DNA sequence and requires no other factors to transcribe a chromatin template. Additionally, T7 polymerase has been used previously as a probe of chromatin accessibility for PcG-repressed regions in vivo (17, 31).

We tested the ability of PRC1 and PCC to repress transcription by T7 RNA polymerase in vitro on both nucleosomal and naked templates (Fig. 4A). As is observed with RNA Pol II transcription, PRC1 and PCC both efficiently inhibit transcription by T7 RNA polymerase on chromatin templates (Fig. 4B and C), and inhibition of transcription on chromatin templates was more efficient than was inhibition on naked DNA (Fig. 4B and C). The concentrations of PcG complexes needed to repress T7 transcription were also similar to those needed to repress transcription by Pol II.

Since T7 RNA polymerase transcription of naked DNA is highly efficient, T7 transcription from a chromatin template might come from a small minority of unassembled templates in the reaction. If this were the case, then PRC1 might repress transcription at a lower concentration in these reactions because the concentration of transcribed templates is lower. To confirm that PRC1 represses transcription from templates that are nucleosomal at the promoter region, we challenged nucleosomal templates with a restriction enzyme before transcription by T7 RNA polymerase (33). When a nucleosome covers the unique *Hha*I site (Fig. 1A), the template is protected from digestion by the restriction enzyme and remains full length. However, templates that do not have a nucleosome covering the *Hha*I site will be cut, and transcription from these cut templates will not be visible by primer extension with a primer downstream of the *Hha*I site. Challenging the template with *Hha*I, then, will eliminate visible transcription from any residual naked templates. It will also eliminate visible transcription from any fully or partially assembled templates that happen not to have a nucleosome positioned over the *Hha*I site, leaving signal only from templates for which the *Hha*I site

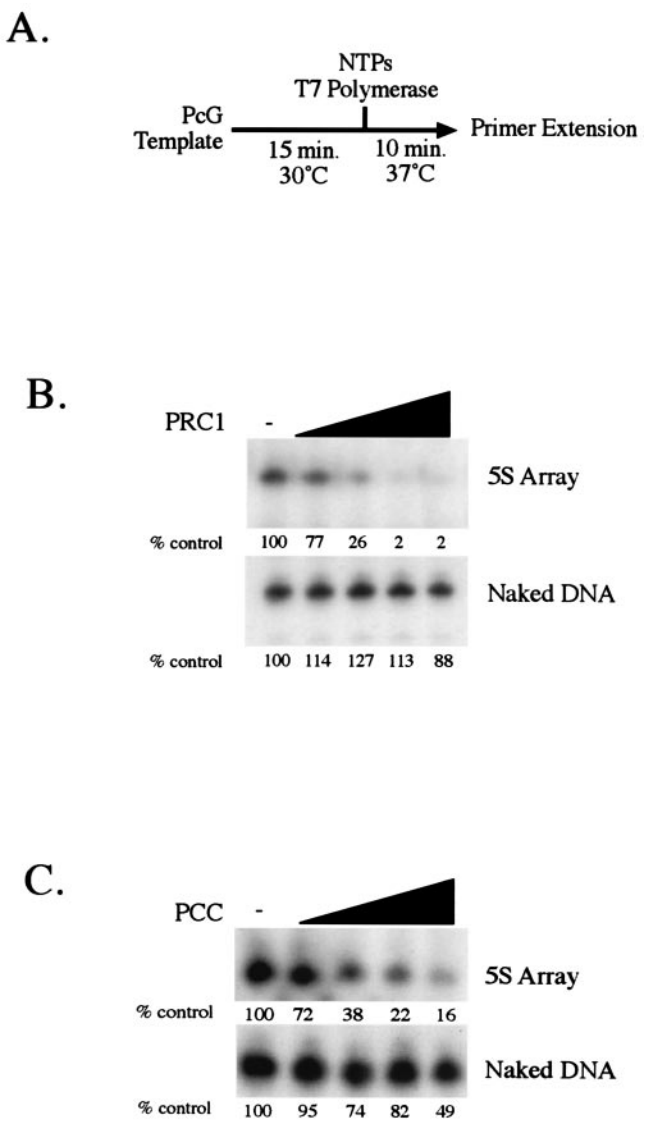

FIG. 4. PRC1 and PCC repress transcription by T7 RNA polymerase. (A) Schematic of reaction protocol for transcription with T7 RNA polymerase. (B and C) PRC1 (B) and PCC (C) were titrated into T7 transcription reaction mixtures. As with Pol II transcription reactions, 20 ng (~15 fmol) of assembled 5S array or 20 ng of unassembled 5S array DNA was used in each reaction. Reaction mixtures contained 2.5, 5, 10, and 20 fmol of PRC1 and 17.5, 35, 70, and 140 fmol of PCC. Quantitation is relative to −PcG lane for each.

is protected. We observed that PRC1 was able to repress transcription of *Hha*I-challenged templates and unchallenged templates to nearly identical levels (Fig. 5), implying that repression of nucleosomal templates was not due to a difference in the concentration of naked templates in the reactions but reflected transcription of nucleosomal templates.

From these experiments we conclude that PRC1 and PCC repress transcription by T7 RNA polymerase and that this repression is chromatin specific to the same degree as is repression of Pol II. Repression of T7 transcription and Pol II transcription required similar ratios of PcG complexes to template. Taken together, these observations are consistent with

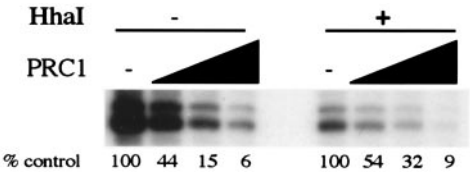

FIG. 5. PRC1 efficiently represses templates challenged with *Hha*I. 5S array templates were cut with *Hha*I before incubation with PRC1 and then transcribed with T7 RNA polymerase. Templates that were nucleosomal at the *Hha*I site adjacent to the T7 start site were protected from digestion, while any residual naked templates were digested. Since transcription was initiated more often from templates in which the T7 start site was unprotected, digesting the template with *Hha*I resulted in a ninefold drop in transcription. Primer extension with the primer used for these reactions gave two products of approximately the expected size. The primer may have recognized two adjacent sequences. Quantitation of transcription products is relative to −PRC1 lane for each set of reactions.

the hypothesis that in vitro repression results not from an interaction with the transcriptional machinery but from an interaction between the PcG complexes and the template.

Preincubation of a template with HeLa nuclear extract precludes repression by PCC. Having established conditions under which we could observe repression by PcG complexes, we asked whether PcG complexes generally inhibit all steps in transcription, or only certain steps. If particular steps are inhibited, then allowing that step to proceed before adding PcG proteins to the reaction might preclude repression. To test this, we changed the order in which components were added to the transcription reaction. These reactions have four steps (Fig. 1E): (i) binding of Gal4-VP16, (ii) remodeling with Swi/Snf, (iii) incubation with HeLa nuclear extract to form a preinitiation complex, and (iv) transcription after the addition of NTPs. We tested whether PCC could inhibit transcription when added before each of these steps.

When PCC was added to the reaction after Gal4-VP16, it was still able to repress transcription as actively as if added

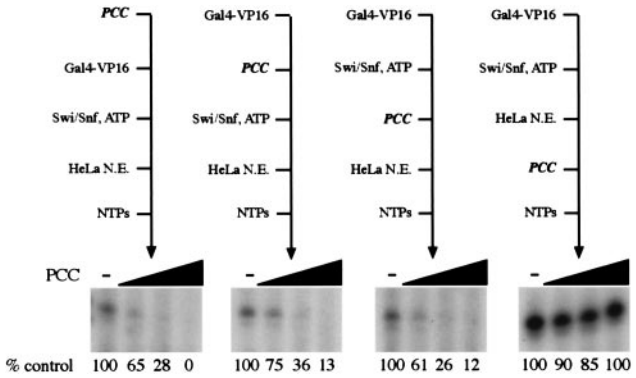

FIG. 6. PCC inhibits transcription when added before HeLa nuclear extract. The order of addition of reaction components was changed as noted. Reactions used 20 ng of 5S nucleosomal array template. PCC was titrated under each reaction protocol (24, 47.5, and 95 fmol). Quantitation is normalized to −PCC lane for each protocol. The transcription signal was sometimes stronger for the PCC-last protocol (fourfold in this case). This may reflect differences in the time that the template was incubated with HeLa nuclear extract (N.E.) before transcription in this protocol.

before Gal4-VP16 (Fig. 6). When PCC was added to the reaction after incubation of the template with Gal4 and with Swi/Snf and ATP, it also was able to inhibit transcription as actively as if added at the beginning of the reaction (Fig. 6). However, when PCC was added to the reaction after the preinitiation complex formation step, it was unable to repress transcription (Fig. 6). We conclude that PCC must be preincubated with the transcription template before HeLa nuclear extract to repress transcription. First, this experiment demonstrates that the presence of PCC in the test tube is not inherently incompatible with transcription. It is thus unlikely that PCC simply removes transcription machinery or the template from solution or that a contaminant in the PCC preparation poisons the reaction or degrades a reaction component. Importantly, this also suggests that PCC might specifically block a step in transcription that occurs during the HeLa nuclear extract incubation, such as preinitiation complex formation.

PRC1 and PCC do not block Gal4-VP16 binding. The results of order-of-addition experiments are consistent with PCC preventing one or more components of the transcriptional machinery from binding to the template. If this is the case, then PCC might prevent all of the proteins necessary for transcription from binding the template, or it might exclude only particular proteins. To determine whether one of these factors could access a template repressed by PcG proteins, we used DNase I footprinting to test directly whether PRC1 and PCC can block Gal4-VP16 from binding the template.

Transcription reaction mixtures with end-labeled 5S array templates were split after a preincubation with PRC1 or PCC followed by a Gal4 binding step. A portion of the reaction mixture was carried through the full transcription protocol. PRC1 and PCC were able to efficiently repress transcription where added (Fig. 7B). The other portion of each reaction mixture was digested with DNase I, producing a distinct footprint at the Gal4 binding sites when Gal4-VP16 was bound to the template (38) (Fig. 7A).

In the absence of PcG proteins, the Gal4-VP16 footprint indicates that the five Gal4 sites are occupied. PRC1 or PCC alone does not change the DNase I digestion pattern of the array template and does not produce a footprint, though the template becomes more sensitive to DNase when PRC1 or PCC is added (see also reference 19). When Gal4-VP16 is added after PRC1 or PCC, a footprint is still present at the Gal4 binding sites, showing that Gal4-VP16 is still able to bind the template in the presence of PRC1 or PCC. In these reactions, then, PCC and PRC1 were able to repress transcription on templates that were bound by Gal4-VP16.

From these experiments we conclude that PRC1 and PCC do not repress transcription in vitro by preventing Gal4-VP16 from accessing a chromatin template. Chromatin incubated with these complexes is permissive for binding of a particular factor but is refractory to one or more subsequent steps in the transcription reaction. Thus, PRC1 and PCC repression are not the result of nonspecific competition with all DNA binding proteins but are specific to certain steps in the transcription reaction.

DISCUSSION

We have established an in vitro system responsive to PcG complexes in order to study PcG repression. We find that, in this system, both PRC1 purified from *Drosophila* embryos and recombinant PCC are able to repress transcription by Pol II and T7 RNA polymerase and that Psc, a subunit of PCC, is able to efficiently repress Pol II transcription on its own, while the other subunits of PCC cannot. PRC1 and PCC appear to repress transcription via an interaction with the transcription template, and they collaborate with nucleosomes to enhance repression when the template is nucleosomal. Notably, though, we find that PcG repression in vitro is not the result of a general block to all DNA binding factors or a prohibitive interaction between the template or the transcription machinery and PcG proteins. The activator Gal4-VP16 is able to bind the template even when transcription is repressed by PRC1 or PCC, and incubation of the template with HeLa nuclear extract prevents repression by PCC.

These results are consistent with the hypothesis that an interaction with the template is primarily responsible for PcG repression, rather than an interaction with the general transcription machinery. PRC1 represses transcription of both RNA Pol II and T7 RNA polymerase, and repression of both of these polymerases is quantitatively similar, suggesting that repression requires neither a specific interaction with either polymerase nor one with eukaryotic transcription factors. In addition, the enhancement of repression that we observe for Pol II transcription on a chromatin template in comparison to naked DNA is also quite similar to the enhancement that we observe with T7 transcription. Since T7 transcription does not involve activators or any of the general transcription factors required for Pol II transcription, it seems unlikely that PRC1 represses transcription predominantly by interacting with or sequestering these factors. The simplest way to explain how PRC1 and PCC behave in this system is that they interact with the template to block steps required for transcription

Though this study indicates that PcG complexes are capable of repressing transcription in vitro through an interaction between the PcG complexes and the template, it is still unknown whether interactions with the general machinery also play a role in PcG repression. Indeed, the finding that PRC1 contains a number of TAF proteins (47), which are also a part of the TFIID complex, raises the possibility of interactions between PRC1 and general factors at the promoter. However, PCC, which contains only PcG proteins, is sufficient for repression in this system, implying that the TAF subunits of PRC1 are not essential for the repression observed here. The TAF subunits may be involved in targeting PcG activity, since TAF proteins are capable of interacting with promoter sequences and with transcription factors (2).

We have found that the Psc subunit of PCC has substantial activity as a single protein, suggesting that Psc may be central to the mechanism of transcriptional repression by PcG complexes. This is in accord with previous results that show that Psc on its own is sufficient to inhibit chromatin remodeling by Swi/Snf in vitro (19) and raises the possibility that a template interaction mediated mainly by Psc is responsible for both of these in vitro activities. The Ph subunit also has a small amount of activity on its own, which is consistent with in vivo studies that have also indicated that Psc and Ph may be central to the mechanism of repression. When Psc and its homolog Su(z)2, or Ph, are removed from imaginal disk clones by recombination, homeotic gene expression is disrupted more quickly and to a

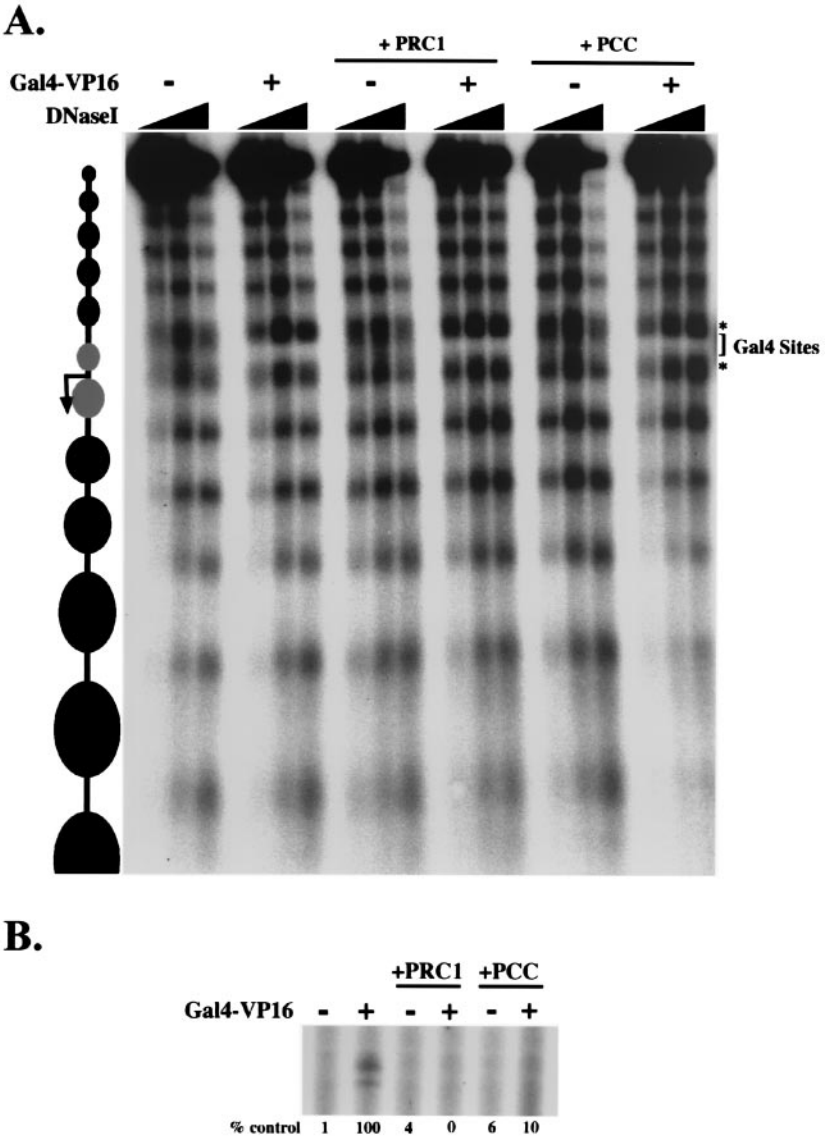

FIG. 7. PRC1 and PCC do not inhibit transcription by blocking the binding of Gal4-VP16. Transcription reaction mixtures containing end-labeled 5S nucleosomal array templates were split, with one portion being analyzed by DNase I footprinting (A) and the other portion being analyzed for transcription (B). Regions that became DNase I hypersensitive upon Gal4 binding are indicated with asterisks, and the region of protection is indicated by the bracket. Reaction mixtures containing PRC1 or PCC used fourfold-lower DNase I concentrations than did the reaction mixtures without PcG complexes (6.25, 12.5, and 25 mU for reaction mixtures with PcG complexes and 25, 50, and 100 mU for reaction mixtures without PcG complexes). Reaction mixtures used 116 fmol of PCC, 150 fmol of PRC1, and 50 ng of 5S array template (3:1 and 4:1 molar ratios, respectively).

greater extent than it is when other PcG genes including Pc are removed (5). The other subunits of PCC that do not appear to inhibit transcription might function primarily in other aspects of repression that are not limiting in this assay, such as the targeting of complexes to specific genes.

Importantly, the proposed interaction between PcG complexes and the transcription template does not seem to constitute an absolute and nonspecific block to all other DNA binding factors. PcG proteins exclude some factors from the template—they inhibit hSwi/Snf binding and remodeling of a PcG-repressed nucleosomal template in vitro (19, 49). On the other hand, Gal4-VP16 is able to bind to the template in reactions where transcription is inhibited. This is also consistent with previous experiments, which showed that templates bound by PRC1 or PCC are open to digestion by micrococcal nuclease, restriction enzymes, and DNase (19, 49). Thus, repression of transcription is not simply the result of general competition between PRC1 or PCC and all DNA binding factors for access to the template.

Our experiments comparing naked DNA and nucleosomal transcription templates provide some evidence that nucleosomes may enhance repression by PcG proteins. It has been proposed elsewhere that PcG and trxG may lock chromatin into particular states, which they are then able to maintain

stably throughout development (18, 40, 52). trxG proteins such as those in the Brahma chromatin-remodeling complex appear to be responsible for establishing an "open" structure that permits gene expression. Conversely, PcG proteins may repress transcription by creating a "closed" chromatin structure that is refractory to transcription. PRC1 and PCC repress transcription quite efficiently in this assay, perhaps reflecting the ability to form a specialized structure that is selectively repressive to protein-DNA interactions. PRC1 and PCC also repress transcription more efficiently on nucleosomal array templates than on naked DNA templates, which might indicate that nucleosomes participate in the interaction between PcG proteins and the template. However, it is important that PRC1 and PCC also repress transcription on naked DNA templates. Interactions between PcG proteins and naked DNA may be important to PcG repression—for instance, the *iab-7* PcG response element of *Drosophila* contains a DNase I-hypersensitive site that can function as a silencing element (32).

Because PcG complexes appear not to block binding of an activator in vitro, they are most likely inhibiting a different step in transcription. Templates that have been preincubated with HeLa nuclear extract cannot be inhibited by PCC, indicating that PcG complexes might not block transcription at the elongation level either. This study raises the possibility that PRC1 and PCC might inhibit transcription by instead blocking some or all of the general transcription machinery and thus might repress transcription by interfering with the formation of the preinitiation complex. Chromatin immunoprecipitation studies with cultured cells have suggested that TBP, TFIIF, and TFIIB are present at PcG-repressed genes even when these genes are repressed, and so it is possible that PcG-repressed chromatin is accessible to a subset of general factors (9). It remains to be seen whether PcG complexes block the association of any or all general transcription factors with the template or whether only certain general transcription factors are able to access the promoter in vitro.

PRC1 and PCC are able to prevent hSwi/Snf from remodeling chromatin templates (19, 49), and this activity might be an important component of the mechanism of transcriptional repression by PcG complexes. However, it is not possible to determine the precise role that inhibition of remodeling plays in this system, since HeLa nuclear extract contains multiple remodeling activities and since the role of remodeling in activating transcription of this template is not completely defined. Chromatin remodeling removes barriers imposed by nucleosomes to any number of steps in transcription, and so it is possible that inhibiting remodeling is sufficient to inhibit transcription. It is also possible, however, that PcG proteins also directly block steps in transcription, such as binding of the general machinery, transcript initiation, or transcript elongation. This hypothesis is supported by the finding that PcG proteins are able to inhibit transcription on naked DNA, where SWI/SNF has no effect on transcription. In remodeling assays preincubation of the template with Swi/Snf is sufficient to prevent repression by PcG complexes, but in transcription assays preincubation with Swi/Snf has little effect on PCC activity. This suggests that PCC might be able to repress transcription by blocking other steps in transcription or by blocking later remodeling events. Further studies using better-defined systems will be necessary to determine the precise role that inhibition of remodeling plays in transcriptional repression by PcG complexes.

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REFERENCES

1. **Akhtar, A., D. Zink, and P. B. Becker.** 2000. Chromodomains are protein-RNA interaction modules. Nature **407:**405–409.
2. **Albright, S. R., and R. Tjian.** 2000. TAFs revisited: more data reveal new twists and confirm old ideas. Gene **242:**1–13.
3. **Bannister, A. J., P. Zegerman, J. F. Partridge, E. A. Miska, J. O. Thomas, R. C. Allshire, and T. Kouzarides.** 2001. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature **410:**120–124.
4. **Bantignies, F., R. H. Goodman, and S. M. Smolik.** 2000. Functional interaction between the coactivator *Drosophila* CREB-binding protein and ASH1, a member of the trithorax group of chromatin modifiers. Mol. Cell. Biol. **20:**9317–9330.
5. **Beuchle, D., G. Struhl, and J. Muller.** 2001. Polycomb group proteins and heritable silencing of Drosophila Hox genes. Development **128:**993–1004.
6. **Boivin, A., and J. M. Dura.** 1998. In vivo chromatin accessibility correlates with gene silencing in Drosophila. Genetics **150:**1539–1549.
7. **Bouazoune, K., A. Mitterweger, G. Langst, A. Imhof, A. Akhtar, P. B. Becker, and A. Brehm.** 2002. The dMi-2 chromodomains are DNA binding modules important for ATP-dependent nucleosome mobilization. EMBO J. **21:**2430–2440.
8. **Breiling, A., E. Bonte, S. Ferrari, P. B. Becker, and R. Paro.** 1999. The *Drosophila* Polycomb protein interacts with nucleosomal core particles in vitro via its repression domain. Mol. Cell. Biol. **19:**8451–8460.
9. **Breiling, A., B. M. Turner, M. E. Bianchi, and V. Orlando.** 2001. General transcription factors bind promoters repressed by Polycomb group proteins. Nature **412:**651–655.
10. **Brown, S. A., C. S. Weirich, E. M. Newton, and R. E. Kingston.** 1998. Transcriptional activation domains stimulate initiation and elongation at different times and via different residues. EMBO J. **17:**3146–3154.
11. **Bunker, C. A., and R. E. Kingston.** 1994. Transcriptional repression by *Drosophila* and mammalian Polycomb group proteins in transfected mammalian cells. Mol. Cell. Biol. **14:**1721–1732.
12. **Carruthers, L. M., C. Tse, K. P. Walker III, and J. C. Hansen.** 1999. Assembly of defined nucleosomal and chromatin arrays from pure components. Methods Enzymol. **304:**19–35.
13. **Czermin, B., G. Schotta, B. B. Hulsmann, A. Brehm, P. B. Becker, G. Reuter, and A. Imhof.** 2001. Physical and functional association of SU(VAR)3–9 and HDAC1 in Drosophila. EMBO Rep. **2:**915–919.
14. **Daubresse, G., R. Deuring, L. Moore, O. Papoulas, I. Zakrajsek, W. R. Waldrip, M. P. Scott, J. A. Kennison, and J. W. Tamkun.** 1999. The Drosophila kismet gene is related to chromatin-remodeling factors and is required for both segmentation and segment identity. Development **126:**1175–1187.
15. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11:**1475–1489.
16. **Dingwall, A. K., S. J. Beek, C. M. McCallum, J. W. Tamkun, G. V. Kalpana, S. P. Goff, and M. P. Scott.** 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.
17. **Fitzgerald, D. P., and W. Bender.** 2001. Polycomb group repression reduces DNA accessibility. Mol. Cell. Biol. **21:**6585–6597.
18. **Francis, N. J., and R. E. Kingston.** 2001. Mechanisms of transcriptional memory. Nat. Rev. Mol. Cell Biol. **2:**409–421.
19. **Francis, N. J., A. J. Saurin, Z. Shao, and R. E. Kingston.** 2001. Reconstitution of a functional core polycomb repressive complex. Mol. Cell **8:**545–556.
20. **Grant, P. A., D. Schieltz, M. G. Pray-Grant, D. J. Steger, J. C. Reese, J. R. Yates III, and J. L. Workman.** 1998. A subset of TAF(II)s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. Cell **94:**45–53.
21. **Hilfiker, A., D. Hilfiker-Kleiner, A. Pannuti, and J. C. Lucchesi.** 1997. mof, a putative acetyl transferase gene related to the Tip60 and MOZ human genes and to the SAS genes of yeast, is required for dosage compensation in Drosophila. EMBO J. **16:**2054–2060.
22. **Ikeda, K., D. J. Steger, A. Eberharter, and J. L. Workman.** 1999. Activation domain-specific and general transcription stimulation by native histone acetyltransferase complexes. Mol. Cell. Biol. **19:**855–863.

23. **Jones, D. O., I. G. Cowell, and P. B. Singh.** 2000. Mammalian chromodomain proteins: their role in genome organisation and expression. Bioessays **22:** 124–137.
24. **Kal, A. J., T. Mahmoudi, N. B. Zak, and C. P. Verrijzer.** 2000. The Drosophila brahma complex is an essential coactivator for the trithorax group protein zeste. Genes Dev. **14:**1058–1071.
25. **Katsani, K. R., J. J. Arredondo, A. J. Kal, and C. P. Verrijzer.** 2001. A homeotic mutation in the trithorax SET domain impedes histone binding. Genes Dev. **15:**2197–2202.
26. **Kennison, J. A.** 1995. The Polycomb and trithorax group proteins of Drosophila: trans-regulators of homeotic gene function. Annu. Rev. Genet. **29:**289–303.
27. **Kennison, J. A., and M. A. Russell.** 1987. Dosage-dependent modifiers of homeotic mutations in *Drosophila melanogaster*. Genetics **116:**75–86.
28. **Kennison, J. A., and J. W. Tamkun.** 1988. Dosage-dependent modifiers of polycomb and antennapedia mutations in Drosophila. Proc. Natl. Acad. Sci. USA **85:**8136–8140.
29. **Lachner, M., D. O'Carroll, S. Rea, K. Mechtler, and T. Jenuwein.** 2001. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature **410:**116–120.
30. **Lewis, E. B.** 1978. A gene complex controlling segmentation in Drosophila. Nature **276:**565–570.
31. **McCall, K., and W. Bender.** 1996. Probes of chromatin accessibility in the Drosophila bithorax complex respond differently to Polycomb-mediated repression. EMBO J. **15:**569–580.
32. **Mishra, R. K., J. Mihaly, S. Barges, A. Spierer, F. Karch, K. Hagstrom, S. E. Schweinsberg, and P. Schedl.** 2001. The *iab-7* Polycomb response element maps to a nucleosome-free region of chromatin and requires both GAGA and Pleiohomeotic for silencing activity. Mol. Cell. Biol. **21:**1311–1318.
33. **Morse, R. H.** 1989. Nucleosomes inhibit both transcriptional initiation and elongation by RNA polymerase III in vitro. EMBO J. **8:**2343–2351.
34. **Muller, J.** 1995. Transcriptional silencing by the Polycomb protein in Drosophila embryos. EMBO J. **14:**1209–1220.
35. **Naar, A. M., B. D. Lemon, and R. Tjian.** 2001. Transcriptional coactivator complexes. Annu. Rev. Biochem. **70:**475–501.
36. **Neely, K., A. Hassan, A. Wallberg, D. Steger, B. Cairns, A. Wright, and J. Workman.** 1999. Activation domain-mediated targeting of the SWI/SNF complex to promoters stimulates transcription from nucleosome arrays. Mol. Cell **4:**649–655.
37. **Ogryzko, V. V., T. Kotani, X. Zhang, R. L. Schlitz, T. Howard, X. J. Yang, B. H. Howard, J. Qin, and Y. Nakatani.** 1998. Histone-like TAFs within the PCAF histone acetylase complex. Cell **94:**35–44.
38. **Owen-Hughes, T., and J. L. Workman.** 1996. Remodeling the chromatin structure of a nucleosome array by transcription factor-targeted trans-displacement of histones. EMBO J. **15:**4702–4712.
39. **Papoulas, O., S. J. Beek, S. L. Moseley, C. M. McCallum, M. Sarte, A. Shearn, and J. W. Tamkun.** 1998. The Drosophila trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. Development **125:**3955–3966.
40. **Paro, R.** 1995. Propagating memory of transcriptional states. Trends Genet. **11:**295–297.
41. **Paro, R., and D. S. Hogness.** 1991. The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of Drosophila. Proc. Natl. Acad. Sci. USA **88:**263–267.
42. **Petruk, S., Y. Sedkov, S. Smith, S. Tillib, V. Kraevski, T. Nakamura, E. Canaani, C. M. Croce, and A. Mazo.** 2001. Trithorax and dCBP acting in a complex to maintain expression of a homeotic gene. Science **294:**1331–1334.
43. **Pirrotta, V.** 1998. Polycombing the genome: PcG, trxG, and chromatin silencing. Cell **93:**333–336.
44. **Poux, S., D. McCabe, and V. Pirrotta.** 2001. Recruitment of components of Polycomb group chromatin complexes in Drosophila. Development **128:**75–85.
45. **Rea, S., F. Eisenhaber, D. O'Carroll, B. D. Strahl, Z. W. Sun, M. Schmid, S. Opravil, K. Mechtler, C. P. Ponting, C. D. Allis, and T. Jenuwein.** 2000. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature **406:**593–599.
46. **Roeder, R. G.** 1996. The role of general initiation factors in transcription by RNA polymerase II. Trends Biochem. Sci. **21:**327–335.
47. **Saurin, A. J., Z. Shao, H. Erdjument-Bromage, P. Tempst, and R. E. Kingston.** 2001. A Drosophila Polycomb group complex includes Zeste and dTAFII proteins. Nature **412:**655–660.
48. **Schlossherr, J., H. Eggert, R. Paro, S. Cremer, and R. S. Jack.** 1994. Gene inactivation in Drosophila mediated by the Polycomb gene product or by position-effect variegation does not involve major changes in the accessibility of the chromatin fibre. Mol. Gen. Genet. **243:**453–462.
49. **Shao, Z., F. Raible, R. Mollaaghababa, J. R. Guyon, C. T. Wu, W. Bender, and R. E. Kingston.** 1999. Stabilization of chromatin structure by PRC1, a Polycomb complex. Cell **98:**37–46.
50. **Sif, S., P. T. Stukenberg, M. W. Kirschner, and R. E. Kingston.** 1998. Mitotic inactivation of a human SWI/SNF chromatin remodeling complex. Genes Dev. **12:**2842–2851.
51. **Simon, J., A. Chiang, and W. Bender.** 1992. Ten different Polycomb group genes are required for spatial control of the abdA and AbdB homeotic products. Development **114:**493–505.
52. **Simon, J. A., and J. W. Tamkun.** 2002. Programming off and on states in chromatin: mechanisms of Polycomb and trithorax group complexes. Curr. Opin. Genet. Dev. **12:**210–218.
53. **Steger, D. J., A. Eberharter, S. John, P. A. Grant, and J. L. Workman.** 1998. Purified histone acetyltransferase complexes stimulate HIV-1 transcription from preassembled nucleosomal arrays. Proc. Natl. Acad. Sci. USA **95:** 12924–12929.
54. **Steger, D. J., and J. L. Workman.** 1999. Transcriptional analysis of purified histone acetyltransferase complexes. Methods **19:**410–416.
55. **Struhl, G., and M. Akam.** 1985. Altered distributions of *Ultrabithorax* transcripts in *extra sex combs* mutant embryos of *Drosophila*. EMBO J. **4:**3259–3264.
56. **Tschiersch, B., A. Hofmann, V. Krauss, R. Dorn, G. Korge, and G. Reuter.** 1994. The protein encoded by the Drosophila position-effect variegation suppressor gene Su(var)3–9 combines domains of antagonistic regulators of homeotic gene complexes. EMBO J. **13:**3822–3831.
57. **Utley, R. T., K. Ikeda, P. A. Grant, J. Cote, D. J. Steger, A. Eberharter, S. John, and J. L. Workman.** 1998. Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. Nature **394:**498–502.
58. **Wedeen, C., K. Harding, and M. Levine.** 1986. Spatial regulation of Antennapedia and bithorax gene expression by the Polycomb locus in Drosophila. Cell **44:**739–748.
59. **Woodage, T., M. A. Basrai, A. D. Baxevanis, P. Hieter, and F. S. Collins.** 1997. Characterization of the CHD family of proteins. Proc. Natl. Acad. Sci. USA **94:**11472–11477.
60. **Workman, J. L., I. C. A. Taylor, R. E. Kingston, and R. G. Roeder.** 1991. Control of class II gene transcription during in vitro nucleosome assembly. Methods Cell Biol. **35:**419–447.
61. **Zawel, L., and D. Reinberg.** 1995. Common themes in assembly and function of eukaryotic transcription complexes. Annu. Rev. Biochem. **64:**533–562.
62. **Zink, D., and R. Paro.** 1995. Drosophila Polycomb-group regulated chromatin inhibits the accessibility of a trans-activator to its target DNA. EMBO J. **14:**5660–5671.