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Stabilization of Chromatin Structure by PRC1, a Polycomb Complex

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Summary

The Polycomb group (PcG) genes are required for maintenance of homeotic gene repression during development. Mutations in these genes can be suppressed by mutations in genes of the SWI/SNF family. We have purified a complex, termed PRC1 (Polycomb repressive complex 1), that contains the products of the PcG genes *Polycomb*, *Posterior sex combs*, *polyhomeotic*, *Sex combs on midleg*, and several other proteins. Preincubation of PRC1 with nucleosomal arrays blocked the ability of these arrays to be remodeled by SWI/SNF. Addition of PRC1 to arrays at the same time as SWI/SNF did not block remodeling. Thus, PRC1 and SWI/SNF might compete with each other for the nucleosomal template. Several different types of repressive complexes, including deacetylases, interact with histone tails. In contrast, PRC1 was active on nucleosomal arrays formed with tailless histones.

Introduction

The expression pattern of the homeotic genes in *Drosophila* is initiated by the gap and pair-rule genes in early embryonic development, and it is regulated throughout development by two groups of genes, the Polycomb group (PcG) and the trithorax group (trxG) (reviewed in Simon, 1995; Cavalli and Paro, 1998; Pirrotta, 1998). PcG genes are involved in repression of the homeotic genes, whereas trxG genes are involved in activation of these genes. There are numerous genes in each group. Classification of a gene as a member of either the PcG or the trxG has been based on phenotype, and it is likely that the members of each group encode multiple complexes with diverse functions. Protein products of each group are believed to act in part by modulating chromatin structure in a manner that creates a repressed (PcG) or an active (trxG) state.

At least 14 PcG genes have been genetically identified (Simon, 1995; Yamamoto et al., 1997), and the products of these genes are believed to form several distinct complexes. Immunolocalization studies have shown that the products of the *Polycomb* (PC), *polyhomeotic* (PH), and *Polycomb-like* (PCL) genes colocalize to numerous specific locations on polytene chromosomes (Franke et al., 1992; Lonie et al., 1994). The products of the *Posterior sex combs* (PSC) and *Sex comb on midleg* (SCM) genes are also found at many of these sites (Martin and Adler, 1993; Peterson et al., 1997). Precipitation studies and yeast two-hybrid interaction experiments support a physical interaction between PC, PH, and PSC (Franke et al., 1992; Strutt and Paro, 1997; Kyba and Brock, 1998), and between SCM and PH (Peterson et al., 1997). These studies support the existence of one large complex that contains PC, PH, and perhaps PCL, PSC, and SCM. Genetic studies indicate that *extra sex combs* (ESC) functions at a different time than PC and PH (Simon et al., 1995), and biochemical studies support an interaction between ESC and the product of *Enhancer of zeste* [E(Z)] (Jones et al., 1998; Tie et al., 1998), suggesting that these proteins are part of a different complex. The only PcG protein with an identified sequence-specific DNA-binding domain is *pleiohomeotic* (PHO), the *Drosophila* homolog of mammalian YY1 (Brown et al., 1998). It is not known which other PcG proteins are contacted by PHO.

Repression by PcG proteins is directed by sequences called PcG response elements (PREs). The *bithorax* complex has served as a model to study this repression (Simon et al., 1993; Chan et al., 1994). There are several PREs in the *bithorax* complex, and PC and PH have been shown by chromosome binding studies and by chromatin immunoprecipitation studies to bind in or near these PRE elements (DeCamillis et al., 1992; Orlando and Paro, 1993; Chiang et al., 1995; Strutt et al., 1997). The nature of the repressed chromatin state that is established by the PcG proteins has been characterized in vivo by examining access of various DNA-binding proteins (Chan et al., 1994; Gindhart and Kaufman, 1995; Zink and Paro, 1995). Transcriptional activation by GAL4 is blocked in chromatin that is under PcG repression, whereas access of T7 RNA polymerase to its promoter is not affected (McCall and Bender, 1996), and there are no large effects on restriction enzyme access (Schlossherr et al., 1994). Two broad classes of mechanisms have been proposed to explain repression by PcG complexes. One hypothesis posits that certain PcG proteins physically spread along the chromatin to produce a repressed state wherever they are present (Paro, 1990); a second hypothesis proposes that PcG proteins are localized to discrete sites, but that they are able to repress access to the DNA over a wide region via mechanisms that do not require continual stable binding of PcG proteins to the repressed region (Pirrotta, 1997).

Genetic interactions between the PcG genes and the trxG genes are consistent with a direct antagonism on chromatin between protein complexes that are encoded by these gene products. Mutations in *brahma* (BRM) suppress PcG mutations in *trans*-heterozygous adults

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(maternal loading effects preclude analysis during embryogenesis) (Kennison and Tamkun, 1988; Tamkun et al., 1992). BRM is a SWI2/SNF2 homolog and is found in a large complex (BRM complex) that has extensive homologies to the SWI/SNF family of ATP-dependent chromatin remodeling complexes (Papoulas et al., 1998). *moira*, another trxG gene that shares phenotypic and genetic characteristics with *brahma* (Kennison and Tamkun, 1988), encodes a protein (MOR) that is the homolog of the yeast SWI3 gene and that is also in the BRM complex (Papoulas et al., 1998; Crosby et al., 1999). The human homologs of BRM and MOR are sufficient for ATP-dependent chromatin remodeling (Phelan et al., 1999), and thus these proteins appear to form a major portion of the catalytic core of SWI/SNF family complexes. Therefore, it is possible that PcG complexes and trxG complexes such as the BRM complex function in opposition to create either a repressed or activated chromatin state on homeotic loci.

To characterize the molecular function of the PcG complexes, it is necessary to purify these complexes. We describe the purification of a complex from *Drosophila* that contains several PcG proteins. This complex directly antagonizes ATP-dependent remodeling of nucleosomal arrays in a purified system and has characteristics that suggest that it and SWI/SNF family complexes can directly modulate each other's activities.

Results

Generation of *Drosophila* Lines that Contain Flag Epitope–Tagged PcG Proteins

To facilitate purification of PcG complexes, we generated fly lines that contained epitope-tagged copies of *polyhomeotic* or *Posterior sex combs*. There are two *ph* genes in *Drosophila* termed proximal *ph* (*ph*-p) and distal *ph* (*ph*-d) that lie immediately adjacent to each other (Hodgson et al., 1997). We constructed P elements that used the Pc promoter to drive expression of N-terminal Flag-tagged PH-p (FPH; Figure 1A) or PSC (FPSC), and we used these P elements to create lines that were homozygous for the tagged transgenes.

Both of the Flag-tagged proteins gave partial rescue of their respective mutant phenotypes. Embryos homozygous for ph^{505} (this *ph* allele is null for both *ph*-p and *ph*-d units [Dura et al., 1987]) lack completely the ventral denticle belts, but the denticle belts are partially restored when the embryos also carry one copy of FPh. Some ph^{505} homozygous embryos carrying two Flag-tagged *ph*-p (FPh) copies showed a full rescue of the denticle belts. Psc^{h27} and Psc^{e24} are loss-of-function alleles (Soto et al., 1995; Wu and Howe, 1995); Psc^{h27}/Psc^{e24} embryos show partial loss of ventral denticles in some belts. Such embryos carrying one copy of Flag-tagged Psc (FPsc) showed a wild-type ventral cuticle, and some of these embryos hatched into larvae. None of the Flag-tagged proteins gave rescue to adulthood. Overall, partial rescue of corresponding mutant phenotypes by the tagged proteins indicates that these fusion proteins interact with the Pc machinery in vivo. The failure to achieve complete rescue may be due to inappropriate expression patterns driven by the *Pc* promoter.

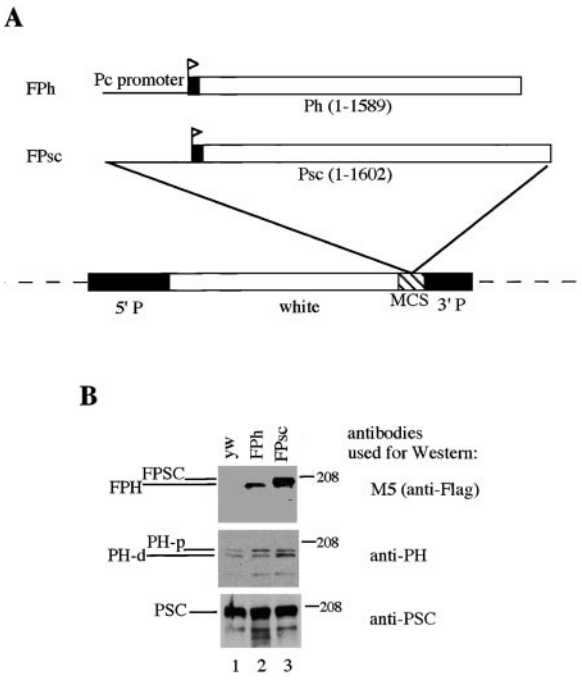

Figure 1. Generation of Transgenic *Drosophila* Lines that Contain Flag-Tagged PH or PSC Protein

(A) Diagram of constructs. Both PH and PSC were tagged at the N termini with Flag sequences (MDYKDDDK) and expressed from an 845 bp Pc promoter. MCS, multiple cloning sites of the vector.

(B) Expression levels of tagged proteins. Nuclear extracts (25 μg) from *y w* (lane 1), FPh71-51A (lane 2), or FPsc46-45A (lane 3) lines were separated by 8% SDS–PAGE, transferred, and probed with antibodies as indicated. The position of a 208 kDa size marker is also indicated.

Lines that expressed tagged PH or tagged PSC from the X chromosome were chosen for further characterization. Antibodies were raised against a region of proximal PH (PH-p; amino acids 753–972) that is highly conserved in distal PH (Hodgson et al., 1997). The affinity-purified antibodies recognized two proteins in nuclear extract (190 and 170 kDa; Figure 1B, middle), which we assigned to PH-p and PH-d based on predicted size. An antibody (M5, Sigma) that specifically recognizes the Flag epitope detected a band in extracts from the FPh line that migrated at the position of PH-p, as expected (Figure 1B, top). The ratio of PH-p to PH-d is higher in the FPh line than in a nontransformed line, as measured using the Ph antibody, suggesting that levels of tagged PH-p are comparable to endogenous levels (compare lane 2 of Figure 1B to lanes 1 and 3). When extracts from the FPsc line were examined, the M5 antibody recognized a band at the position of PSC (Figure 1B, top), and there was no detectable increase in total PSC expression (Figure 1B, bottom).

Fractionation and Purification of Pc Complexes

We initially expanded a FPh line for purification because of its relatively high level expression of tagged PH. Nuclear extract was fractionated using Biorex 70 chromatography (Figures 2A and 2B). Most (~75%) of the FPH and PSC proteins eluted at 0.85 M KCl (Figure 2B), and

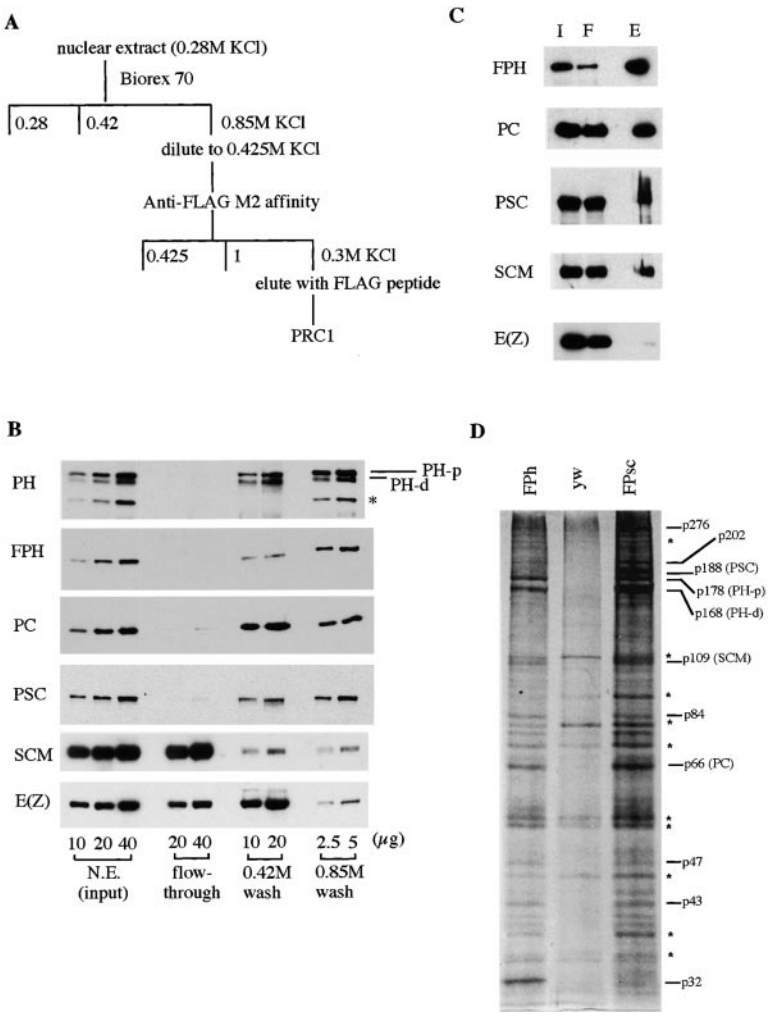

Figure 2. Purification of *Drosophila* PRC1 Complex

(A) Scheme for the fractionation and purification of *Drosophila* PRC1.

(B) Western Blots of the PcG members following fractionation of extract from the FPh71-51A line on Biorex 70. Antibodies used to probe each blot are indicated (left). The band labeled with an asterisk in the PH analysis was not recognized by a different PH antibody provided by A. Peterson and Dr. J. Simon, so it might be the result of cross-reaction. A monoclonal antibody against the Flag epitope (M5, Sigma) was used to detect FPH.

(C) PC, PSC, and SCM coelute with FPH from M2-affinity column. Input (I; 5 μg), flow-through (F; 5 μg), and the peak elution (E; 1 μl, ~10 ng) were immunoblotted using the indicated antibodies. Approximately 50% of FPH binds to the beads; FPH represents approximately one-third of the PH in the extract, so we expect ~15% of PC to bind to the beads, and this is seen.

(D) Composition of FPH and FPSC complexes. Peak eluates (15 μl) of M2 affinity columns purified from FPh, FPsc, or parental *y w* lines according to (A) were separated by 8% SDS–PAGE and silver stained. Lines mark proteins found consistently only in the tagged lines, and asterisks mark proteins also found in the mock purification from *y w*.

PC was also enriched in this fraction. (Note that in the Western analysis of Figure 2B lower amounts of protein were analyzed as salt concentration increased in order to keep the signal in a similar range of intensity.) The majority of SCM did not bind to Biorex 70 in the presence of 0.28 M KCl, while the remaining SCM protein was eluted in both the 0.42 M and 0.85 M wash. In addition, both E(Z) and PCL proteins are primarily enriched in the 0.42 M wash (Figure 2B and data not shown). These data demonstrate that Biorex 70 chromatography differentially separates PcG proteins, indicating that there are multiple complexes that contain PcG proteins.

We chose to further purify the PH-containing complexes from the 0.85 M salt wash, as this fraction contained most of the PH protein. This fraction was bound to an M2 affinity column (Kodak) at 0.425 M salt, the column was washed extensively with 1 M salt, reequilibrated to 0.3 M salt, and eluted with Flag peptide. Western blot analysis (Figure 2C) showed that FPH (and PH-p and PH-d, data not shown), PSC, PC, and SCM proteins all copurified in this step, and that approximately 400-fold purification was achieved. E(Z), PCL, GAGA, and dCtBP proteins did not copurify (Figure 2C and data not shown). An analogous "mock" purification was performed from the parental *y w* line, following exactly the same scheme as described. The protein composition of the M2 fractions from tagged extracts was compared to the mock-purified complex from *y w* lines in several different experiments; we identified 11 peptides that were consistently enriched in the tagged preparations (Figure 2D, lines), five of which migrate at the position of PSC, PH-p, PH-d, SCM, and PC.

The PSC protein copurified with FPH in the fractionation described above. To address whether these proteins might be part of similar/identical complexes, we used the scheme described above to purify a PSC-containing complex from extracts from a transgenic line that expressed FPSC. The complex purified via tagged PSC had a composition that was similar to that of the complex purified via tagged PH (Figure 2D; note that the intensities of all proteins are somewhat higher in the FPSC lane, and that PSC is enriched in this lane). This indicates that PSC and PH are found in similar or identical complexes. Complexes purified via FPH or via FPSC behaved identically in all of the functional analyses reported below. We will refer to the complex purified from either line as PRC1 (Polycomb repressive complex 1).

Based on Western analysis, the complexes that we have isolated over the M2 column are 3000- to 4000-fold purified over nuclear extract, and they are approximately

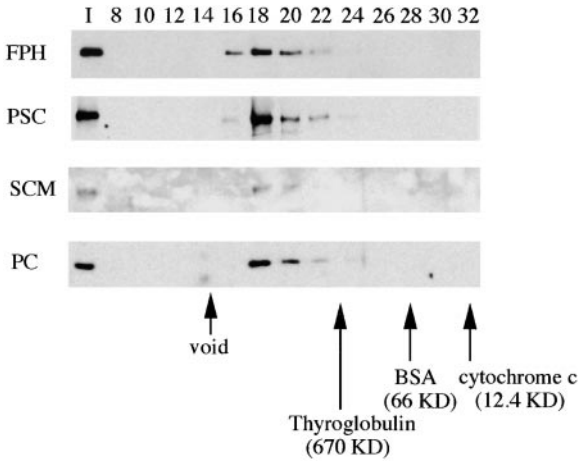

Figure 3. PRC1 Migrates as a Large Complex on a Sizing Column

M2 eluates (200 μl) from FPh and 100 μg of cytochrome c (20 mg/ml) were applied to a Sephacryl S-400 column (0.7 × 30 cm). Two hundred microliter fractions were collected until cytochrome c ran out of the column, and 17.5 μl per fraction was analyzed by Western blot with indicated antibodies. Due to the lack of a suitable large size marker, the void volume and sizes were estimated from the manufacturer's specifications.

50% pure based on silver staining (Figure 2D). We analyzed these fractions further using fractionation on standard sizing resins. This resulted in reproducibly low yields; however, all detectable FPH, PC, SCM, and PSC ran in the void volume of Superose 6 and migrated at approximately 2–6 MDa on Sephacryl S-400HR (Figure 3A and data not shown). Silver stain analysis of these fractions showed that all of the peptides identified as being in the PRC1 complex in Figure 2D coeluted (data not shown).

Inhibition of Remodeling

Little is known about the mechanism through which PcG proteins maintain a repressed state of transcription. One hypothesis is that PcG proteins form a complex that creates a stably repressed chromatin structure. Genetic analyses of *brahma* and *moira* suggest that PcG genes and these trxG genes function at the same mechanistic level but in opposite directions (Kennison and Tamkun, 1988). Both BRM and MOR are members of a large complex that is apparently homologous to members of the SWI/SNF family of ATP-dependent chromatin remodeling complexes (Papoulas et al., 1998; Crosby et al., 1999). These observations led us to test whether PRC1 was capable of stabilizing chromatin arrays against the actions of ATP-dependent remodeling complexes.

One hallmark of the SWI/SNF complexes is the ability to alter DNA topology in an ATP-dependent manner (Guyon et al., 1999). Assembly of a plasmid template into nucleosomes introduces one negative supercoil per nucleosome, which is visualized when the DNA is deproteinized and electrophoresed. When a 3.2 kb plasmid is assembled into nucleosomes, the resultant introduction of ~16 negative supercoils causes the DNA to run rapidly on a native agarose gel (e.g., Figure 4A, lane 1). Addition of a human SWI/SNF and ATP in the presence of Topoisomerase I causes a significant decrease in the number of supercoils (lane 2), indicating an ATP-dependent change in the topology of the plasmid.

We first tested whether PRC1 (M2 fraction, purified via FPH) was able to suppress this remodeling activity. To circumvent any requirement for targeting of PRC1 via PREs, which almost certainly requires other complexes in addition to PRC1, we used a single plasmid template in this reaction so that PRC1 was targeted by mass action. This approach has been successful with other chromatin modifying complexes (reviewed in Workman and Kingston, 1998). Preincubation of the template with PRC1 blocked the ability of the template to be remodeled (Figure 4A, lanes 3 and 4), while preincubation with the mock-purified fraction from *y w* lines had little effect (lanes 5 and 6; the PRC1 and *y w* fractions used here were those shown in Figure 2D, FPh and *y w* lanes). The inhibitory activity of PRC1 on hSWI/SNF-mediated remodeling activity was lost when the complex was heat inactivated at 65°C for 20 min (data not shown) and occurred at levels that we estimate to be below one complex per nucleosome (see legend).

We next determined whether inhibition of remodeling by PRC1 required either preincubation with the DNA template or preincubation with SWI/SNF. Inhibition of remodeling was observed when PRC1 was preincubated with the DNA template for as little as 5 min (Figure 4B, lanes 3–5), but it did not occur if the complex was added at the same time as SWI/SNF (lane 6) or was preincubated with SWI/SNF (data not shown and see below). Increasing amounts of SWI/SNF in the reaction did not affect the extent of inhibition by PRC1, and complete inhibition of remodeling was observed even at ratios (by weight) of 30-fold excess of SWI/SNF over PRC1 (Figure 4C). (Weight ratios are likely to be similar to molar ratios as SWI/SNF is ~2 MDa in size.)

As the remodeling of arrays by SWI/SNF under these conditions is not complete until ~20–45 min after the start of the reaction (data not shown), we were intrigued by the observation that addition of SWI/SNF and PRC1 at the same time resulted in no repression by PRC1. This indicated that a template that was complexed with SWI/SNF was impervious to subsequent repression by PRC1; thus, SWI/SNF and PRC1 might compete with each other for the template.

Establishment of a repressed state by PRC1 does not require ATP hydrolysis. Preincubation of the template with PRC1 either in the presence or absence of ATP completely repressed remodeling by SWI/SNF (Figure 5B, compare lane 3 to lane 6). The ATP-independent nature of PRC1 function allowed us to perform experiments to determine whether the presence of ATP (required for SWI/SNF function) would affect competition between SWI/SNF and PRC1 for template. We added SWI/SNF and PRC1 to the template at the same time and then waited for varying amounts of time before adding ATP to the reaction (Figure 5A). There was no repression seen when PRC1 and SWI/SNF were preincubated for up to 30 min with template in the absence of added ATP (lanes 4–7). Thus, PRC1 was only able to inhibit remodeling if present prior to SWI/SNF, and addition of SWI/SNF blocked the ability of PRC1 to form this repressed state even when SWI/SNF was inactive due to the absence of ATP.

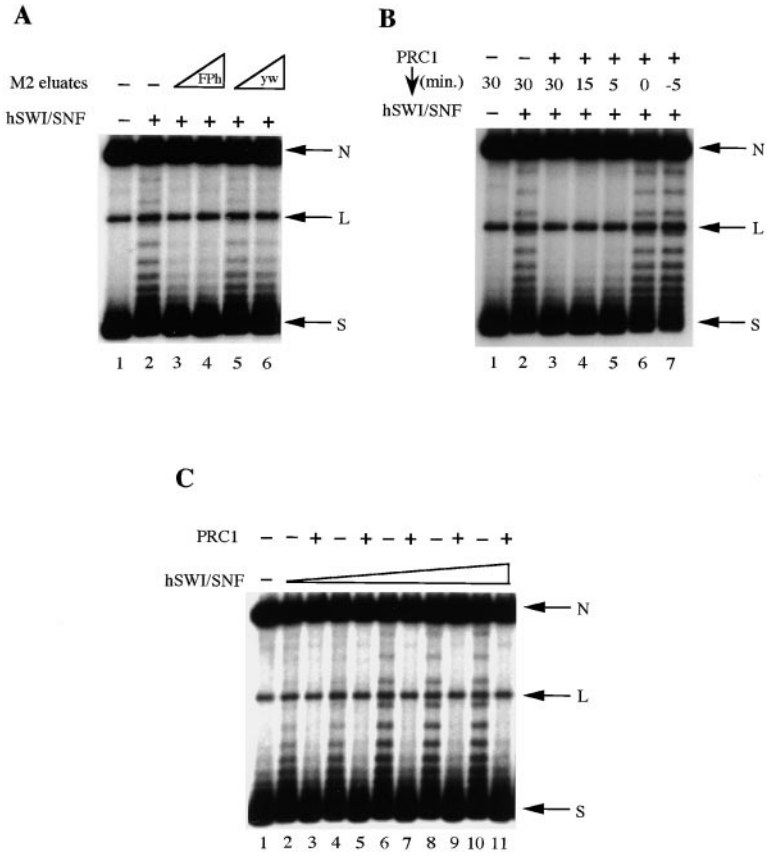

Figure 4. PRC1 Inhibits hSWI/SNF Remodeling Activity on Plasmid Nucleosomal Templates

(A) Eluates of affinity columns from FPh lines, but not from *y w* lines, inhibit SWI/SNF remodeling activity. M2 eluates from FPh or *y w* lines (the precise fractions shown in Figure 2D) were used. One or two microliters (~10 ng/μl) of FPh (lanes 3 and 4) or 1 or 2 μl of the paired *y w* extract (lanes 5 and 6) was incubated with nucleosomal template (1 ng), Topoisomerase I (0.2 U), and 2 mM ATP at 30°C for 30 min. Two microliters of hSWI/SNF (50 ng/μl) was added, and the reactions proceeded at 30°C for 60 min before deproteinization and analysis by nondenaturing electrophoresis. N, nicked DNA; L, linear DNA; S, supercoiled DNA.

(B) PRC1 needs to bind to the template first in order to inhibit SWI/SNF remodeling activity. Twenty-five nanograms of PRC1 (FPH) was added to the reactions either before or after the addition of hSWI/SNF for the indicated times. Reactions were stopped 60 min after the addition of hSWI/SNF.

(C) Increased amount of hSWI/SNF could not overcome PRC1-mediated repression. Reaction templates were preincubated with or without PRC1 (FPH, 20 ng) as indicated. Increasing amounts of hSWI/SNF were then added to the reactions as follows: 33 ng (lanes 2 and 3), 100 ng (lanes 4 and 5), 200 ng (lanes 6 and 7), 400 ng (lanes 8 and 9), and 800 ng (lanes 10 and 11).

We conclude that PRC1 does not directly inactivate the SWI/SNF complex, because mixing these two complexes together for extended times did not inactivate SWI/SNF (Figure 5A). In addition, there was no detectable modification of the SWI/SNF complex by PRC1 under repressive conditions, as judged by silver-stained SDS–PAGE gels (data not shown). Furthermore, under identical conditions used for the experiments reported above, PRC1 was not able to repress alterations in DNase I digestion pattern of mononucleosomes mediated by SWI/SNF (data not shown). Thus, inhibition of remodeling appears to involve an interaction of PRC1 with the template.

Histone Tails Are Not Required for Repression

Histone tails have been implicated in the formation of repressive complexes with the SIR proteins and have been postulated to provide a molecular "handle" for such complexes (reviewed in Grunstein, 1998). They are also the site of histone acetylation and are thus targets for complexes that function as histone acetyltransferases or as deacetylases. Histone tails are not required for remodeling by the SWI/SNF family of complexes (Guyon et al., 1999; Logie et al., 1999), so we were able to test whether tails are required for the ability of PRC1 to repress this remodeling.

Chromatin templates were assembled with histones

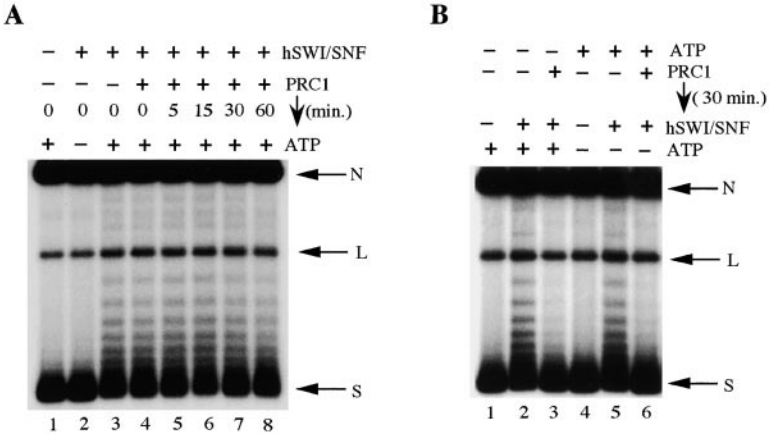

Figure 5. PRC1 Does Not Directly Inhibit hSWI/SNF Activity

(A) hSWI/SNF complex was still active after preincubation with PRC1. Both PRC1 (FPH, 30 ng) and hSWI/SNF complexes (100 ng) were incubated with the templates for indicated times before the addition of 2 mM ATP. The reactions then proceeded at 30°C for another 60 min in the presence of ATP.

(B) ATP is not required for PRC1 function. Templates were incubated with PRC1 (30 ng) in the absence (lanes 1–3) or presence (lanes 4–6) of ATP at 30°C for 30 min, then hSWI/SNF (150 ng) with (lanes 1–3) or without (lanes 4–6) 2 mM ATP was added to the reactions for 60 min at 30°C.

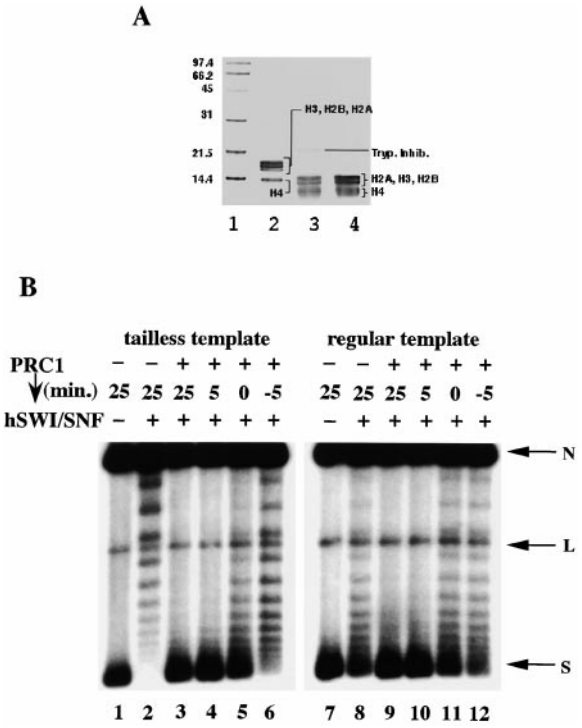

Figure 6. Histone Tails Are Not Required for PRC1-Mediated Inhibition

(A) SDS–PAGE analysis of trypsinized histones used in assembling tailless templates (from Guyon et al., 1999). Lane 1, size standards indicated in kilodaltons. Lane 2, H1-depleted nucleosomes prior to trypsinization. Lane 3, nucleosomes after trypsinization (Tryp Inhib, trypsin inhibitor). Lane 4, histones purified after hydroxyapatite chromatography.

(B) Plasmid templates were packaged into nucleosomes with either tailless histones (lanes 1–6) or intact histones (lanes 7–12). The reactions were similar to Figure 4B, except that the PRC1 used here was purified from a FPsc line.

that had been treated with trypsin to remove their N-terminal tails (Figure 6A). PRC1 (M2 fraction purified via FPSC) repressed remodeling by SWI/SNF to a similar extent on tailed and tailless templates (Figure 6B). In contrast to what was seen on tailed templates, addition of PRC1 and SWI/SNF at the same time to tailless templates did result in some repression of remodeling (compare lane 5 to lanes 2 and 6), suggesting that removal of the tails alters the balance between PRC1 and SWI/SNF in favor of PRC1.

Discussion

Understanding the mechanism by which the Polycomb group proteins repress gene expression requires the isolation of functional PcG complexes. We have purified a PcG complex, termed PRC1, and demonstrated that PRC1 is capable of stabilizing a nucleosomal array to the effects of ATP-dependent remodeling by SWI/SNF. This inhibition of SWI/SNF-mediated remodeling activity does not require ATP (Figure 5B), requires preincubation of the template with PRC1 (Figure 4B), and cannot be overcome by increasing the amount of SWI/SNF (Figure 4C). PRC1 is unlikely to modify SWI/SNF directly, as preincubation of PRC1 with SWI/SNF either with or without template does not inactivate remodeling (Figures 4 and 5). Taken together, these results suggest that PRC1 functions by interacting with nucleosomal arrays to stabilize them against subsequent remodeling.

PRC1 and SWI/SNF can block each other's function. PRC1 blocks SWI/SNF function only if it is added to nucleosomal arrays before SWI/SNF. If PRC1 and SWI/SNF are added at the same time, PRC1 is not able to establish a repressed state. The ability of SWI/SNF to prevent PRC1 repression does not require ATP (Figure 5) and hence does not require remodeling activity. One simple hypothesis is that PRC1 and SWI/SNF bind to identical or overlapping faces on the nucleosome, and that SWI/SNF has a greater affinity for this site. Steric interference between SWI/SNF and PRC1 (both of which are considerably larger than a nucleosome) could also account for the competition between these two complexes. PRC1 appears to form a repressive complex with nucleosomal arrays at a ratio of less than one complex per nucleosome (see Figure 4A legend). Each PRC1 complex may have multiple binding sites for nucleosomes, thereby allowing one complex to stabilize a large region of nucleosomal DNA via contacts with each nucleosome. Alternatively, PRC1 might use "looping" to bind at a small number of sites in a manner that stabilizes all of the intervening nucleosomes to SWI/SNF action.

Histone N-terminal tails, the sites for acetylation and deacetylation, are not required for function of PRC1 on nucleosomal arrays (Figure 6). The ability of PRC1 to function on tailless templates distinguishes this complex from repressive complexes such as deacetylase complexes or the SIR complex (Hecht et al., 1995), and it implies that PRC1 must interact with either the main body of the nucleosomal proteins or with nucleosomal DNA. Consistent with these data, PRC1 contains very low (close to background) levels of histone deacetylase activity (data not shown). SWI/SNF also does not require tails for function (Guyon et al., 1999; Logie et al., 1999), and thus these antagonistic complexes might have similar mechanisms of interacting with nucleosomes. The lack of a requirement for histone tails in forming a repressive state does not preclude a role for tails in the targeting of PcG proteins in vivo. In addition, histone deacetylation may be employed as a marker for the repressed state through cell proliferation (Pirrotta, 1998).

Composition of PRC1 and Heterogeneity of PcG Complexes

It is apparent from the composition of PRC1 that there must be other PcG complexes in addition to PRC1. PRC1 purified via either tagged PH or PSC contains PC, PSC, PH-p, PH-d, and SCM, as well as several other proteins (Figure 2D). PRC1 does not contain PCL and E(Z) (Figure 2 and data not shown). Previous studies using immunoprecipitation, in vitro binding, and/or yeast two-hybrid analysis have shown that PC, PSC, and PH interact with each other (Strutt and Paro, 1997; Kyba and Brock, 1998), and that SCM interacts with PH (Peterson et al., 1997). E(Z) and ESC have been shown to interact with each other by similar approaches (Jones et al., 1998; Tie et al., 1998), and E(Z) separates from PRC1 during chromatography (Figure 2C). Similarly,

mammalian homologs of PcG can also be separated into roughly two complexes, one containing homologs to PC, PSC, and PH (Alkema et al., 1997; Satijn et al., 1997), and the other containing homologs to E(Z) and ESC (Sewalt et al., 1998; van Lohuizen et al., 1998). Another argument that E(Z) and ESC form a separate complex with a distinct function is based on the observation that homologs to these genes are found in the *C. elegans* genome (Holdeman et al., 1998; Korf et al., 1998), whereas homologs to *Pc*, *Ph*, or *Psc* are not.

The activities of PRC1 suggest that it may be directly involved in creating the repressed state, and that it may require other complexes for targeting. Through screens for homeotic derepression, 14 PcG genes have been well characterized genetically (Simon, 1995; Yamamoto et al., 1997). It is possible that a subset of these genes are required for direct repression, while other PcG proteins function in targeting, regulation of repression activity, or maintenance of the repressed state through mitosis. How PcG proteins are recruited to their targets is still unknown, but several proteins have been suggested as candidates for this function, such as ESC and E(Z) (Struhl and Brower, 1982; Rastelli et al., 1993), and sequence-specific DNA-binding proteins PHO (Brown et al., 1998), GAGA (Strutt et al., 1997), *HUNCHBACK* (HB) (Zhang and Bienz, 1992), and the HB interacting protein dMi-2 (Kehle et al., 1998). PRC1 does not contain E(Z) (Figure 2C) and GAGA (data not shown). Using antibodies against a region of human YY1 that is conserved in PHO (Santa Crutz, C-20), we find that PHO is unlikely to be in PRC1 (data not shown); an antibody made specifically against PHO is needed to verify this result. Due to the lack of antibodies, we have not tested whether PRC1 contains ESC, HB, or dMi-2.

Immunostaining data have shown that PCL colocalizes completely with PH and PC on polytene chromosomes (Lonie et al., 1994); however, PRC1 does not contain PCL (data not shown). PCL is, therefore, not absolutely required for repression of remodeling by PRC1. One possibility is that PCL plays a role in targeting PRC1; a second possibility is that PCL is normally associated with PRC1 in vivo, but its interaction is not strong enough to survive the purification procedure.

Competition between PRC1 and SWI/SNF

PcG proteins are conserved from flies to mammals, and SWI/SNF family complexes have been identified and characterized in yeast (SWI/SNF and RSC), *Drosophila* (BRM complex), and humans (the hSWI/SNF family; Papoulas et al., 1998, and references therein). All SWI/SNF complexes share a set of conserved proteins, and the yeast and human complexes have ATP-dependent remodeling activities whose tested characteristics appear identical. We show here that *Drosophila* PRC1 can stabilize a template against subsequent remodeling by hSWI/SNF, and that hSWI/SNF can block PRC1 function. This implies either that there are required contacts between PRC1 and SWI/SNF that are conserved from flies to mammals, or that PRC1 organizes the nucleosomal array into a stabilized state that blocks a conserved mechanism for SWI/SNF-dependent remodeling.

Organisms that contain the PcG proteins of PRC1 have highly differentiated tissues. These organisms might have acquired PRC1 function during evolution to block chromatin remodeling by the SWI/SNF family and thereby maintain a repressed state over genes that are involved in specifying differentiated states (similar to arguments made by Bird, 1995). It is intriguing that PRC1 must be present first on the template to block SWI/SNF function. It is possible that appropriate regulation of genes throughout development requires a mechanism that favors SWI/SNF unless PRC1 has already established a repressed state. The SWI/SNF complexes function catalytically, and thus their function on a given region of chromatin does not require continual occupancy of any given nucleosome. The competitive "advantage" that SWI/SNF appears to have in our in vitro studies might provide a means of blocking PRC1 from binding and repressing regions of chromatin where SWI/SNF is active but not continually present. The function of recruitment might be to bring PRC1 to the template at a time when SWI/SNF is not present. The challenge now will be to establish conditions in vitro where PRC1 repression is dependent on the DNA sequence of a Polycomb response element.

Experimental Procedures

Plasmid Constructions

The vector pCaSpeR4 was used to construct each P element plasmid. Plasmid PFPh71 has a 845 bp Pc promoter fragment (1–841) (Paro and Hogness, 1991), a PCR-generated sequence encoding the Flag tag (MDYKDDDK), and a cDNA encoding amino acids 1–1589 of proximal PH, all inserted between Kpn I and Xba I sites of pCaSpeR4. Plasmid FPsc46 was similar, except that it included amino acids 1–1602 of PSC. A vector containing 1–390 of PC (FPc19) was also made, and lines were created with this vector and characterized genetically, although purification was not performed from these lines. All of the cloning junctions were sequenced. Detailed descriptions of these plasmids are available upon request. GST-Pc (191–380) and GST-Ph (752–972) were constructed by PCR amplification of corresponding sequences and insertion into EcoR I and Xho I sites of pGEX-KG.

P Element–Mediated Germline Transformation

To introduce the constructed plasmids into *Drosophila melanogaster*, P element–mediated germline transformation (Rubin and Spradling, 1982) was carried out using the pπ25.7wc helper plasmid (Karess and Rubin, 1984). For the FPc, FPh, and FPsc constructs, 7, 12, and 14 independent lines were generated, respectively. Homozygous stocks were established for the majority of these transgenic lines. The FPh and FPsc stocks used here were homozygous for the transgenes.

Genetic Rescue Experiments

For Pc and Psc rescue experiments, females homozygous for a Flag-tagged insert and carrying one copy of a corresponding mutant allele (Pc^3 and Psc^{h27}, respectively) were crossed to males carrying one copy of a different mutant allele of the same gene (Pc^2 and Psc^{e24}, respectively). Results from the Pc rescue experiments are available upon request. For the ph rescue experiments, males homozygous for either one or two copies of selected FPh inserts were crossed to females carrying the null ph^{505} allele. Therefore, in the rescue attempts, all F1 progeny received one copy of the Flag-tagged insert (or two copies for some of the ph tests), with one-quarter of them also carrying two mutant alleles of the corresponding gene. The control crosses were the same as the experimental ones except that no Flag-tagged insert was present. The percentage of F1 embryos that failed to hatch was compared for each rescue attempt and its corresponding control cross. In each case, the phenotypic features of those experimental and control embryos that failed to hatch were compared. Adults collected from each cross

were examined for balancer chromosome markers to detect potentially rescued genotypes.

Purification of PRC1 from Transgenic *Drosophila* Embryos

Embryos (0–12 hr) were harvested and stored for up to 3 days at 4°C. Purification steps were performed at 4°C unless otherwise indicated. Nuclear extracts were prepared according to Kamakaka et al. (1991) with some modification. Embryos were homogenized by a motor-driven pestle in buffer I (15 mM HEPES, K^+ [pH 7.6], 10 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM EGTA, 350 mM sucrose, 1 mM dithiothreitol [DTT], 1 mM benzamidine-HCl, 0.5 mM phenylmethysulfonyl fluoride [PMSF]). The nuclei pellets were washed once with buffer I and were subsequently resuspended in buffer C (20 mM HEPES, K^+ [pH 7.9], 420 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 25% glycerol, 0.5 mM DTT, 0.5 mM PMSF, 1 μg/ml of aprotinin and leupeptin, and 50 μg/ml of TLCK, at 1.8 ml/g nuclei) for 30 min with rotation, before being subjected to centrifugation at 42,000 rpm for 1 hr in a Beckman SW55Ti rotor. The salt concentration of the supernatant (nuclear extract) was approximately 0.28 M KCl. Typically 360–400 mg of the extract was then fractionated on a 40 ml Biorex 70 (Biorad) column, which was preequilibrated with BC buffer (20 mM HEPES, K^+ [pH 7.9], 0.2 mM EDTA, 20% glycerol, 0.5 mM DTT, 0.5 mM PMSF) containing 0.28 M KCl, and washed with the same buffer until absorbance at 280 nm was down to basal level. The wash was followed with 0.42 M and 0.85 M KCl elution steps, and the fractions containing the majority of the protein were pooled for each step. After dilution with an equal volume of HEGN buffer (25 mM HEPES, K^+ [pH 7.6], 0.1 mM EDTA, 10% glycerol, 0.1% NP-40, 1 mM DTT, 0.1 mM PMSF) without KCl, the pooled 0.85 M KCl fractions (~36 mg) were incubated with anti-Flag M2 agarose resin (1.1 ml, Kodak) for 16 hr by rotation. The resin was then packed into a column, washed extensively with 10 column volumes of 0.425-HEGN buffer (HEGN plus 0.425 M KCl), 60 column volumes of 1-HEGN buffer (HEGN with 1 M KCl), and 10 column volumes of 0.3-HEGN buffer. The bound proteins were eluted by 0.3-HEGN buffer containing 0.4 mg/ml of the Flag peptide (DYKDDDDK, MGH peptide synthesis facility) and aprotinin (1 μg/ml), leupeptin (1 μg/ml), and TLCK (50 μg/ml). For analysis on sizing columns, PRC1 purified through M2 affinity chromatography (200 μl) was loaded on Superose 6 or Sephacryl S-400 HR (both from Pharmacia; 11 ml) in 0.15-HEGN plus 50 μg/ml insulin. Two hundred microliters of fractions was analyzed by Western blot analysis. Protein markers were cytochrome c (12.4 kDa), BSA (66 kDa), and Thyroglobulin (670 kDa). Blue dextran 2000 was used to determine the void volume of the Superose 6 column.

Western Blotting and Antibody Production

Proteins were analyzed by standard SDS–PAGE, transferred to nitrocellulose membranes, and detected by ECL reagents according to the manufacturer's recommendations (Amersham). Pc and Ph rabbit polyclonal antisera were raised against GST-PC (191–390) and GST-PH (753–972), respectively, by Covance, Inc. Both antibodies were affinity purified against GST fusions covalently linked to Sepharose 4B, following manufacturer's procedure (Pharmacia). A second Pc antibody was kindly provided by Dr. P. O'Farell. The region of PH-p used to raise our Ph antibody is conserved between PH-p and PH-d, and a second Ph polyclonal antibody (kindly provided by A. Peterson and Dr. J. Simon; raised against PH residues 88–341, which are also conserved between PH-p and PH-d) also recognizes the same two bands on Western blots. Other antibodies employed here are as follows: rabbit polyclonal antibodies against SCM (kindly provided by Dr. J. Simon), E(Z) (Dr. R. Jones), PCL (Dr. R. Saint), and YY1 (Santa Cruz Biotechnology, C-20); rat polyclonal antibody against dCtBP (Dr. Y. Nibu and Dr. M. Levine); and mouse monoclonal antibodies against PSC (Dr. P. Adler) and Flag epitope (Sigma, M2 and M5).

Chromatin Remodeling Assays

HeLa histones or trypsinized histones, as well as hSWI/SNF, were purified as described (Guyon et al., 1999). A 3.35 kb plasmid, pG5HC2AT, was internally labeled and assembled into nucleosomal template using a *Xenopus* heat-treated extract (Guyon et al., 1999), in order to avoid contamination of the reactions with other *Drosophila* proteins. Remodeling assays were performed in 12 mM HEPES, K^+ (pH 7.9), 60 mM EDTA, 4 mM $MgCl_2$, 2 mM ATP/$MgCl_2$, 0.2 U of Topoisomerase I (Promega), and 60 mM KCl. Glycerol gradient-purified nucleosomal template (1–2 ng of DNA) was incubated with affinity-purified PRC1 for 30 min at 30°C before the addition of hSWI/SNF complex for another 60 min. Reactions were then stopped with addition of one-third of the reaction volume of stop buffer (50 mM Tris [pH 8.0], 0.1 mM EDTA, 25% glycerol, 3% SDS, 2 mg/ml proteinase K), then incubated at 37°C for at least 1 hr before they were analyzed on a 1.8% agarose gel. Similar results were obtained following template assembly with *Drosophila* S-190 extracts (Kamakaka et al., 1993); however, inhibition of remodeling was sometimes not as efficient as was seen with *Xenopus* assembled templates (data not shown).

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References

Alkema, M.J., Bronk, M., Verhoeven, E., Otte, A., van't Veer, L.J., Berns, A., and van Lohuizen, M. (1997). Identification of Bmi1-interacting proteins as constituents of a multimeric mammalian polycomb complex. Genes Dev. *11*, 226–240.

Bird, A.P. (1995). Gene number, noise reduction and biological complexity. Trends Genet. *11*, 94–100.

Brown, J.L., Mucci, D., Whiteley, M., Dirksen, M.L., and Kassis, J.A. (1998). The Drosophila Polycomb group gene pleiohomeotic encodes a DNA binding protein with homology to the transcription factor YY1. Mol. Cell *1*, 1057–1064.

Cavalli, G., and Paro, R. (1998). Chromo-domain proteins: linking chromatin structure to epigenetic regulation. Curr. Opin. Cell Biol. *10*, 354–360.

Chan, C.S., Rastelli, L., and Pirrotta, V. (1994). A Polycomb response element in the Ubx gene that determines an epigenetically inherited state of repression. EMBO J. *13*, 2553–2564.

Chiang, A., O'Connor, M.B., Paro, R., Simon, J., and Bender, W. (1995). Discrete Polycomb-binding sites in each parasegmental domain of the bithorax complex. Development *121*, 1681–1689.

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.

DeCamillis, M., Cheng, N.S., Pierre, D., and Brock, H.W. (1992). The polyhomeotic gene of Drosophila encodes a chromatin protein that shares polytene chromosome-binding sites with Polycomb. Genes Dev. *6*, 223–232.

Dura, J.M., Randsholt, N.B., Deatrick, J., Erk, I., Santamaria, P., Freeman, J.D., Freeman, S.J., Weddell, D., and Brock, H.W. (1987). A complex genetic locus, polyhomeotic, is required for segmental specification and epidermal development in D. melanogaster. Cell *51*, 829–839.

Franke, A., DeCamillis, M., Zink, D., Cheng, N., Brock, H.W., and Paro, R. (1992). Polycomb and polyhomeotic are constituents of a multimeric protein complex in chromatin of Drosophila melanogaster. EMBO J. *11*, 2941–2950.

Gindhart, J.G., Jr., and Kaufman, T.C. (1995). Identification of Polycomb and trithorax group responsive elements in the regulatory region of the Drosophila homeotic gene Sex combs reduced. Genetics *139*, 797–814.

Grunstein, M. (1998). Yeast heterochromatin: regulation of its assembly and inheritance by histones. Cell *93*, 325–358.

Guyon, J.R., Narlikar, G.J., Sif, S., and Kingston, R.E. (1999). Stable remodeling of tailless nucleosomes by the human SWI-SNF complex. Mol. Cell. Biol. *19*, 2088–97.

Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S.M., and Grunstein, M. (1995). Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell *80*, 583–592.

Hodgson, J.W., Cheng, N.N., Sinclair, D.A., Kyba, M., Randsholt, N.B., and Brock, H.W. (1997). The polyhomeotic locus of Drosophila melanogaster is transcriptionally and post-transcriptionally regulated during embryogenesis. Mech. Dev. *66*, 69–81.

Holdeman, R., Nehrt, S., and Strome, S. (1998). MES-2, a maternal protein essential for viability of the germline in Caenorhabditis elegans, is homologous to a Drosophila Polycomb group protein. Development *125*, 2457–2467.

Jones, C.A., Ng, J., Peterson, A.J., Morgan, K., Simon, J., and Jones, R.S. (1998). The Drosophila esc and E(z) proteins are direct partners in polycomb group-mediated repression. Mol. Cell. Biol. *18*, 2825–2834.

Kamakaka, R.T., Tyree, C.M., and Kadonaga, J.T. (1991). Accurate and efficient RNA polymerase II transcription with a soluble nuclear fraction derived from Drosophila embryos. Proc. Natl. Acad. Sci. USA *88*, 1024–1028.

Kamakaka, R.T., Bulger, M., and Kadonaga, J.T. (1993). Potentiation of RNA polymerase II transcription by Gal4-VP16 during but not after DNA replication and chromatin assembly. Genes Dev. *7*, 1779–1795.

Karess, R.E., and Rubin, G.M. (1984). Analysis of P transposable element functions in Drosophila. Cell *38*, 135–146.

Kehle, J., Beuchle, D., Treuheit, S., Christen, B., Kennison, J.A., Bienz, M., and Muller, J. (1998). dMi-2, a hunchback-interacting protein that functions in polycomb repression. Science *282*, 1897–1900.

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.

Korf, I., Fan, Y., and Strome, S. (1998). The Polycomb group in Caenorhabditis elegans and maternal control of germline development. Development *125*, 2469–2478.

Kyba, M., and Brock, H.W. (1998). The Drosophila polycomb group protein Psc contacts ph and Pc through specific conserved domains. Mol. Cell. Biol. *18*, 2712–2720.

Logie, C., Tse, C., Hansen, J.C., and Peterson, C.L. (1999). The core histone N-terminal domains are required for multiple rounds of catalytic chromatin remodeling by the SWI/SNF and RSC complexes. Biochemistry *38*, 2514–2522.

Lonie, A., D'Andrea, R., Paro, R., and Saint, R. (1994). Molecular characterisation of the Polycomblike gene of Drosophila melanogaster, a trans-acting negative regulator of homeotic gene expression. Development *120*, 2629–2636.

Martin, E.C., and Adler, P.N. (1993). The Polycomb group gene Posterior Sex Combs encodes a chromosomal protein. Development *117*, 641–655.

McCall, K., and Bender, W. (1996). Probes of chromatin accessibility in the Drosophila bithorax complex respond differently to Polycomb-mediated repression. EMBO J. *15*, 569–580.

Orlando, V., and Paro, R. (1993). Mapping Polycomb-repressed domains in the bithorax complex using in vivo formaldehyde cross-linked chromatin. Cell *75*, 1187–1198.

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.

Paro, R. (1990). Imprinting a determined state into the chromatin of Drosophila. Trends Genet. *6*, 416–421.

Paro, R., and Hogness, D.S. (1991). The Polycomb protein shares a homologous domain with a heterochromatin-associated protein of Drosophila. Proc. Natl. Acad. Sci. USA *88*, 263–267.

Peterson, A.J., Kyba, M., Bornemann, D., Morgan, K., Brock, H.W., and Simon, J. (1997). A domain shared by the Polycomb group proteins Scm and ph mediates heterotypic and homotypic interactions. Mol. Cell. Biol. *17*, 6683–6692.

Phelan, M.L., Sif, S., Narlikar, G.J., and Kingston, R.E. (1999). Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. Mol. Cell *3*, 247–253.

Pirrotta, V. (1997). Chromatin-silencing mechanisms in Drosophila maintain patterns of gene expression. Trends Genet. *13*, 314–318.

Pirrotta, V. (1998). Polycombing the genome: PcG, trxG, and chromatin silencing. Cell *93*, 333–336.

Rastelli, L., Chan, C.S., and Pirrotta, V. (1993). Related chromosome binding sites for zeste, suppressors of zeste and Polycomb group proteins in Drosophila and their dependence on Enhancer of zeste function. EMBO J. *12*, 1513–1522.

Rubin, G.M., and Spradling, A.C. (1982). Genetic transformation of Drosophila with transposable element vectors. Science *218*, 348–353.

Satijn, D.P., Gunster, M.J., van der Vlag, J., Hamer, K.M., Schul, W., Alkema, M.J., Saurin, A.J., Freemont, P.S., van Driel, R., and Otte, A.P. (1997). RING1 is associated with the polycomb group protein complex and acts as a transcriptional repressor. Mol. Cell. Biol. *17*, 4105–4113.

Schlossherr, J., Eggert, H., Paro, R., Cremer, S., and Jack, R.S. (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.

Sewalt, R.G., van der Vlag, J., Gunster, M.J., Hamer, K.M., den Blaauwen, J.L., Satijn, D.P., Hendrix, T., van Driel, R., and Otte, A.P. (1998). Characterization of interactions between the mammalian polycomb-group proteins Enx1/EZH2 and EED suggests the existence of different mammalian polycomb-group protein complexes. Mol. Cell. Biol. *18*, 3586–3595.

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

Simon, J., Chiang, A., Bender, W., Shimell, M.J., and O'Connor, M. (1993). Elements of the Drosophila bithorax complex that mediate repression by Polycomb group products. Dev. Biol. *158*, 131–144.

Simon, J., Bornemann, D., Lunde, K., and Schwartz, C. (1995). The extra sex combs product contains WD40 repeats and its time of action implies a role distinct from other Polycomb group products. Mech. Dev. *53*, 197–208.

Soto, M.C., Chou, T.B., and Bender, W. (1995). Comparison of germline mosaics of genes in the Polycomb group of Drosophila melanogaster. Genetics *140*, 231–243.

Struhl, G., and Brower, D. (1982). Early role of the esc+ gene product in the determination of segments in Drosophila. Cell *31*, 285–292.

Strutt, H., and Paro, R. (1997). The polycomb group protein complex of Drosophila melanogaster has different compositions at different target genes. Mol. Cell. Biol. *17*, 6773–6783.

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.

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

Tie, F., Furuyama, T., and Harte, P.J. (1998). The Drosophila Polycomb Group proteins ESC and E(Z) bind directly to each other and co-localize at multiple chromosomal sites. Development *125*, 3483–3496.

van Lohuizen, M., Tijms, M., Voncken, J.W., Schumacher, A., Magnuson, T., and Wientjens, E. (1998). Interaction of mouse polycomb-group (Pc-G) proteins Enx1 and Enx2 with Eed: indication for separate Pc-G complexes. Mol. Cell. Biol. *18*, 3572–3579.

Workman, J.L., and Kingston, R.E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu. Rev. Biochem. *67*, 545–579.

Wu, C.-t., and Howe, M. (1995). A genetic analysis of the Suppressor 2 of zeste complex of Drosophila melanogaster. Genetics *140*, 139–181.

Yamamoto, Y., Girard, F., Bello, B., Affolter, M., and Gehring, W.J. (1997). The cramped gene of Drosophila is a member of the Polycomb-group, and interacts with mus209, the gene encoding Proliferating Cell Nuclear Antigen. Development *124*, 3385–3394.

Zhang, C.C., and Bienz, M. (1992). Segmental determination in Drosophila conferred by hunchback (hb), a repressor of the homeotic gene Ultrabithorax (Ubx). Proc. Natl. Acad. Sci. USA *89*, 7511–7515.

Zink, D., and Paro, R. (1995). Drosophila Polycomb-group regulated chromatin inhibits the accessibility of a trans-activator to its target DNA. EMBO J. *14*, 5660–5671.