The Human Polycomb Group Complex Associates with Pericentromeric Heterochromatin to Form a Novel Nuclear Domain

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Abstract. The Polycomb group (PcG) complex is a chromatin-associated multiprotein complex, involved in the stable repression of homeotic gene activity in *Drosophila*. Recently, a mammalian PcG complex has been identified with several PcG proteins implicated in the regulation of Hox gene expression. Although the mammalian PcG complex appears analogous to the complex in *Drosophila*, the molecular mechanisms and functions for the mammalian PcG complex remain unknown. Here we describe a detailed characterization of the human PcG complex in terms of cellular localization and chromosomal association. By using antibodies that specifically recognize three human PcG proteins—RING1, BMI1, and hPc2—we demonstrate in a number of human cell lines that the PcG complex forms a unique discrete nuclear structure that we term PcG bodies. PcG bodies are prominent novel nuclear structures with the larger PcG foci generally localized near the centromeres, as visualized with a kinetochore anti-

body marker. In both normal fetal and adult fibroblasts, PcG bodies are not randomly dispersed, but appear clustered into defined areas within the nucleus. We show in three different human cell lines that the PcG complex can tightly associate with large pericentromeric heterochromatin regions (1q12) on chromosome 1, and with related pericentromeric sequences on different chromosomes, providing evidence for a mammalian PcG–heterochromatin association. Furthermore, these heterochromatin-bound PcG complexes remain stably associated throughout mitosis, thereby allowing the potential inheritance of the PcG complex through successive cell divisions. We discuss these results in terms of the known function of the PcG complex as a transcriptional repression complex.

Key words: Polycomb-group • RING finger protein • centromere • heterochromatin • repression

ORPHOLOGICAL differences among segments in *Drosophila* are controlled by spatially restricted expression of homeotic genes from the *Bithorax* and *Antennapedia* gene complexes (BX-C and Ant-C, respectively; Kaufman et al., 1980; Lewis, 1978). These patterns of expression are established early in *Drosophila* embryonic development by transiently expressed transcriptional regulators encoded by the segmentation genes (for review see Bienz and Müller, 1995). The spatial patterns and levels of homeotic gene expression are, however, maintained throughout the rest of development by two

groups of different genes named after their founding members: *trithorax* (*trxG*) and *Polycomb* (*PcG*) (for reviews see Kennison, 1995; Orlando and Paro, 1995; Pirrotta, 1997; Pirrotta, 1998; Simon, 1995). The trithorax group (*trxG*) acts as a transcriptional activator of homeotic genes, while the Polycomb group (*PcG*) acts as a transcriptional repressor. TrxG and PcG proteins are expressed throughout the embryo, consistent with their acting to maintain spatially restricted gene expression previously established by other factors (Franke et al., 1992; Martin and Adler, 1993; Zink and Paro, 1993; reviewed in Bienz and Müller, 1995).

Although the PcG proteins have long been established as being the principle mediators of *Drosophila* homeotic gene silencing, exactly how they achieve this is still unclear (for reviews see Bienz and Müller, 1995; Kennison, 1995; Pirrotta, 1998; Simon, 1995; Singh, 1994). One can con-

sider the mechanism of PcG gene silencing in two parts: (*a*) recruitment to and formation of the PcG multiprotein complex at specific chromosomal loci; and (*b*) molecular consequences due to the binding of a PcG protein complex. A recruitment mechanism was suggested when *Polycomb* (*Pc*) fused to the GAL4 DNA-binding domain was shown to silence a reporter gene containing GAL4 DNA target sites in *Drosophila* embryos (Müller, 1995). Furthermore, the silencing required other PcG proteins, indicating that targeted Pc can recruit other PcG proteins, and that the PcG complex has to be anchored to DNA through specific *cis*-regulatory sequences (Müller, 1995). Another possibility is that the formation of the PcG complex arises through multiple weak and low-specificity DNA–protein interactions by several PcG proteins at particular clustered binding sites (Pirrotta, 1997). Nonetheless, PcG complexes in *Drosophila* are known to bind to *cis*-acting DNA elements of the homeotic genes, termed PcG response elements (PREs),[1] although no conserved sequence element has as yet been identified (Chan et al., 1994; Gindhart and Kaufman, 1995; Müller and Bienz, 1991; Simon et al., 1993). The importance of such PREs in mediating repression has been demonstrated by inserting PREs into reporter gene constructs in transgenic flies. Here, additional Pc binding was observed on these transgenes, as well as silencing of the neighboring reporter genes (Chan et al., 1994; Zink et al., 1991; Zink and Paro, 1995).

Recently a growing number of mammalian homologues of *Drosophila* PcG and trxG have been identified (Alkema et al., 1997; Gunster et al., 1997; Satijn et al., 1997*b*; for reviews see Gould, 1997; Schumacher and Magnuson, 1997). When combined with the increasing genetic evidence of related homeotic gene loci in mammals (*Hox* genes), a functional and evolutionary similarity in homeotic gene control between mammals and *Drosophila* is apparent (Krumlauf, 1994; reviewed in Gould, 1997; Schumacher and Magnuson, 1997). The mammalian *Hox* genes play a key role in vertebrate development, as judged by the loss or gain of function in mutant mice, although little is known about regulation of *Hox* gene expression (Krumlauf, 1994). Identification of mammalian trxG and PcG homologues has allowed the study of Hox expression patterns and vertebrate development in knockout mice for several of these components (reviewed in Gould, 1997). In addition to controlling anterior/posterior cell fate, it appears that the mammalian PcG/trxG system also regulates blood cell differentiation and proliferation (reviewed in Schumacher and Magnuson, 1997).

We first described the cloning and sequencing of a previously unknown human gene localizing proximal to the major histocompatibility complex on chromosome 6, which we called RING1 (Lovering et al., 1993). RING1 led to the identification of a novel zinc-binding motif, the RING finger, which has now been found in nearly 80 proteins from differing sources and functions (Saurin et al., 1996). Recently, RING1 was shown to interact directly in vivo with a human protein with homologies to Pc (hPc2), a human polyhomeotic homologue (HPH1), and human BMI1 (Satijn, et al., 1997*b*). Furthermore, RING1 is able to repress transcription of reporter constructs in a dose-dependent manner, and colocalizes to human PcG complexes in tissue culture cells, strongly suggesting that RING1 is part of a mammalian PcG complex (Satijn et al., 1997*b*). A mouse homologue (Ring1A) has recently been isolated, and has been shown to repress transcription of reporter genes, interact with a mouse homologue of Pc (M33), and colocalize with other known mammalian PcG proteins in discrete nuclear foci (Schoorlemmer et al., 1997). A *Drosophila* RING1 has recently appeared in the cDNA database, and thus awaits deletion analysis to fully place it amongst the PcG family by the classical definition.

In these and more recent studies on PcG protein distribution in developing *Drosophila* embryos (Buchenau et al., 1998), it is clear that generic PcG multiprotein complexes form discrete nuclear foci (as observed by indirect immunofluorescence) of differing composition, number, and size, in interphase nuclei. To further understand the nature and any functional significance of these nuclear domains in mammalian cells, we have studied the spatial organization of three human PcG proteins in several human cell lines using antibodies to human RING1, BMI1, and hPc2. We show that in a variety of transformed and primary cell lines, the human PcG complex forms discrete nuclear foci that are unrelated to any other known nuclear domain. We find a direct heterochromatin association of the PcG complex in interphase cells, which in three cell lines tested, corresponds to pericentromeric DNA sequences on chromosome 1 and related pericentromeric sequences on other chromosomes. Furthermore, this association is maintained throughout cell division, suggesting a possible mechanism for the inheritance of PcG complexes by subsequent daughter cells. Our data provides the first detailed evidence for mammalian PcG-pericentromeric heterochromatin association, an association which has also recently been observed for one PcG component (PSC) in developing *Drosophila* embryos (Buchenau et al., 1998).

Materials and Methods

Production of Polyclonal Antisera to RING1

Two polyclonal antisera against human RING1 were raised by immunizing rabbits with either bacterially expressed recombinant RING1 protein or a fifteen-residue synthetic RING1 peptide. The polyclonal antibody ASA3 was raised from rabbits immunized with the full-length RING1 protein expressed in *Escherichia coli* BL21 cells as a His6-tagged fusion protein according to the pET expression system protocol (Novagen, Inc., Madison, WI). The polyclonal antibody ASA8 was raised by immunizing rabbits with a synthetic RING1 peptide (residues 142–156) coupled to Keyhole Limpet Hemocyanin (Sigma Chemical Co., St. Louis, MO) according to Reichlin (1980). A further antipeptide antibody, ASA10, was made against a synthetic peptide (residues 274–288). All antibodies raised gave the same immunofluorescent signal, and specificity for RING1 was determined as described in Results.

Immunofluorescence Microscopy

Unless otherwise stated, cells were grown to near confluency on glass coverslips and washed with PBS before fixing. Two fixation protocols were used for all experiments. These were either 4% formaldehyde for 10 min followed by permeabilizing with PBS containing 0.5% Triton X-100 for 5 min, or methanol at −20°C for 10 min. After rehydration in PBS, cells were blocked with PBS containing 10% newborn serum (NBS; GIBCO

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1. *Abbreviations used in this paper*: FISH, fluorescence in situ hybridization; NBS, newborn serum; PREs, PcG response elements; PTB, polypyrimidine tract-binding protein.

BRL, Gaithersburg, MD) for 10 min at room temperature. Antibody incubations were performed in PBS containing 10% NBS, 0.1% Tween 20 for 1 h at room temperature. After three 5-min washes in PBS, samples were incubated with fluorochrome-conjugated secondary antibodies in PBS containing 10% NBS, 0.1% Tween 20 for 30 min. Coverslips were then washed 1× for 5 min in PBS containing 0.1% Tween 20, 3× for 5 min in PBS, followed by 1× for 5 min in dH$_2$O before mounting and visualization. Secondary antibodies used were swine anti–rabbit FITC-conjugate (1:200; DAKO Corp., Carpinteria, CA) or sheep anti–mouse Texas red conjugate (1:200; Amersham Corp., Arlington Heights, IL). DNA staining was achieved by incubating samples for 3 min in PBS containing 0.4 mg/ml Hoechst 33258 (Sigma Chemical Co.) before mounting. All samples were visualized and recorded on an MRC 1000 (Bio-Rad Laboratories, Hercules, CA) or a DM IRBE TCS-NT inverted confocal microscope (Leica Inc., Deerfield, IL).

Combined Technique (Immuno-Fluorescence In Situ Hybridization [FISH])

To combine detection of proteins by immunofluorescence and DNA by FISH (immuno-FISH), cells were either grown on slides or cytocentrifuged onto slides using a cyto-tek centrifuge at 200 rpm for 10 min. Cells were then fixed and permeabilized as described above, incubated for at least 1 h in 20% glycerol in PBS, followed by five cycles of freeze/thawing in liquid nitrogen (Kurz et al., 1996). After immunolabeling, cells were refixed in 4% formaldehyde for 10 min to help preserve antibody signal during the subsequent in situ hybridization procedures. Cells were denatured in 70% formamide, 2× SSC, pH 7.0 at 72°C for 2 min, dehydrated through an ethanol series, and air-dried. Whole chromosomes were detected using biotin-labeled chromosome-specific paints (Cambio Ltd., Cambridge, United Kingdom), while the pericentromeric heterochromatin of chromosome 1 was detected using the plasmid clone pUC1.77 (Cooke, 1979) labeled by nick translation using digoxigenin 11-dUTP (Boehringer Mannheim Corp., Indianapolis, IN). Labeled probe DNAs were precipitated and resuspended in 50% (moderate hybridization stringency) or 30% (low hybridization stringency) deionized formamide, 2× SSC, 10% dextran sulfate. After denaturation at 75°C for 5 min, probe DNA was applied to the denatured cells, sealed, and incubated overnight at 37°C in a humidified chamber. Posthybridization washes were performed at 42°C in 50% formamide, 2× SSC, pH 7.0, 3× for 5 min followed by 2× SSC for 3× for 5 min (moderate hybridization stringency) or at room temperature in 2× SSC 3× for 5 min (low hybridization stringency). Nonspecific signal was blocked by incubation in 4× SSC, 0.1% Tween 20, 5% low-fat dried milk for 10 min at 37°C. Biotin-labeled probes were detected using avidin-conjugated Texas red (Vector Labs, Inc., Burlingame, CA), and digoxigenin-labeled probes were detected using rhodamine-conjugated antidigoxigenin antibody (Boehringer Mannheim Corp.). Images were captured using a Axioscop microscope (Carl Zeiss Inc., Thornwood, NY) equipped with a CCD camera (Photometrics Ltd., Tucson, AZ), or on an MRC 1000 inverted confocal microscope (Bio-Rad Laboratories).

Visualization of Mitotic Chromosomes

Condensed chromosomes were visualized by propidium iodide staining of fixed cells. Optimizing the fixation conditions used allowed visualization of antigens throughout cell division. Cells on coverslips were fixed using a standard protocol in 4% formaldehyde for 20 min before permeabilization with 0.5% Triton X-100 for 10 min. This fixation allowed detection of antigens at some stages of mitosis. Fixing cells in 1.5% formaldehyde (methanol-free, EM grade; TAAB Laboratories, England) before permeabilizing as described above allowed visualization of all antigens studied at all (or most) stages of mitosis. After this procedure, all washes were performed carefully so as not to dislodge mitotic cells. To decrease cytoplasmic background from RNA, the cells were treated with RNase A (1 mg/ml in PBS, 10 mM MgCl$_2$; Boehringer Mannheim Corp.) for 30 min at room temperature before incubating with the primary antibody as described above. Propidium iodide (Sigma Chemical Co.) was added to the secondary antibody solution at a final concentration of 0.1 µg/ml, and was incubated for 30 min at room temperature. Rhodamine filters were used to detect propidium iodide-stained cells on a DM IRBE TCS-NT inverted confocal microscope (Leica Inc.).

Antibodies

Antibodies against the following antigens were used during this study: RING1 (ASA3 and ASA8), hPc2 and BMI1 (Satijn et al., 1997a; Satijn et al.,

1997b), PML to recognize PML nuclear bodies (5E10; Stuurman et al., 1992), SMN recognizing gems (2B1; kindly provided by G. Dreyfuss of Liu and Dreyfuss, 1996), p80 coilin recognizing coiled bodies (pseudo-δ; kindly provided by A.I. Lamond, Department of Biochemistry, University of Dundee, Dundee, Scotland), polypyrimidine tract-binding protein (PTB) recognizing PTB-containing complexes (kindly provided by D. Spector, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; Huang et al., 1997), Sm antigen recognizing all snRNP proteins (Y-12; kindly provided by J. Steitz, Department of Molecular Biophysics and Biochemistry, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT), CENP-B and CENP-C (kindly provided by W.C. Earnshaw, Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, Scotland), kinetochore complexes (H33; described in Stuurman et al., 1992), M31 and M32 (MAC353 and MAC358; kindly provided by P. Singh, Department of Development and Genetics, Babraham Institute, Cambridge, United Kingdom), MeCP2 (kindly provided by A.P. Bird, Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, Scotland), GAGA factor (Z13-3; kindly provided by J.W. Raff, Wellcome/CRC Institute, Department of Genetics, Cambridge, United Kingdom), SC-35 (Sigma Chemical Co.), proliferating cell nuclear antigen (PCNA) recognizing sites of DNA replication (PC10; Sigma Chemical Co.), BrUTP to recognize BrUTP-incorporated sites of active trancription (anti-BrdUTP; Boehringer Mannheim Corp.) and SP-1 (1C6; Santa Cruz Biotechnology, Santa Cruz, CA).
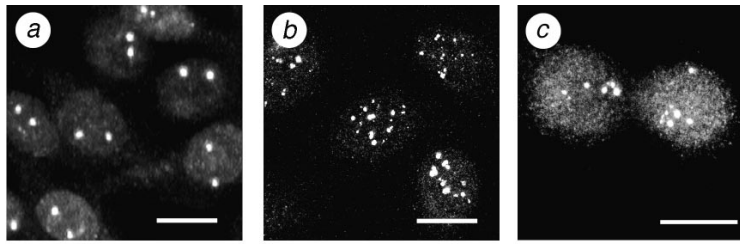
Results

The RING1 Protein Localizes to the Nucleus in Discrete Foci in a Number of Cell Lines

In several previous studies, RING1-staining nuclear foci in human U-2 OS and SW480 cell lines were shown to contain the human PcG proteins BMI1, hPc2, HPH1, and HPH2 as well as RING1 (Gunster et al., 1997; Satijn et al., 1997b). Furthermore, several studies have shown direct in vivo interactions between human RING1, hPc2, BMI1, HPH1, and HPH2 (Gunster et al., 1997; Satijn et al., 1997b), murine Bmi1, Mel18, M33, and MPh/RAE28 (Alkema et al., 1997; Hashimoto et al., 1998), and between *Xenopus* Pc and bmi-1 (Reijnen et al., 1995).

To characterize in detail the subcellular localization of human RING1, we raised polyclonal antisera against full-length recombinant RING1 protein, and against two 15-residue synthetic peptides (data not shown). The immunofluorescent signal was the same for all the antisera with the specificity of the antibody for RING1, shown by specifically blocking the antipeptide antisera with the immunizing peptide, resulting in total loss of the immunofluorescent signal (data not shown). There was no loss of immunofluorescent signal when the antisera was blocked using a nonimmunizing RING1 peptide. Antibody specificity was further demonstrated by the recognition of in vitro–translated RING1 and RING1 from whole-cell extracts by immunoblot analyses (data not shown). We then performed double immunofluorescent labeling experiments with RING1 and antibodies against BMI1 and hPc2 (Satijn et al., 1997a; Satijn et al., 1997b) in a variety of transformed and primary human cell lines including 2C4 (HT1080 derivative), U-2 OS, SAOS-2, HEP-2, SW480, T24, HeLa, A431, MRC-5, WI-38, CS22F, and frozen human foreskin tissue sections (Fig. 1 and data not shown).

In all cell lines tested, we found that RING1 localizes to the nucleus, and is often concentrated into discrete foci that vary in number and size (Fig. 1 and data not shown). In 2C4 (HT1080) cells, the signal is very prominent and
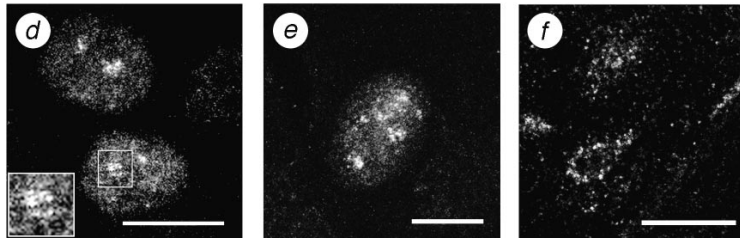
highly concentrated into two foci between 1 and 1.5 μm in size (by fluorescence signal) per nucleus (Fig. 1 *a*). In U-2 OS cells, the number of RING1-staining domains increases to between six and fourteen per nucleus, and their relative sizes differ from 0.2 to 1.5 μm (Fig. 1 *b*). RING1 is also concentrated into foci in SAOS-2 cells, although in this cell line the foci are smaller (on average ~0.5 μm) with between four and seven present in each nucleus (Fig. 1 *c*). In nontransformed cells with known and normal karyotypes, RING1 staining gives a microgranular signal comprising numerous small nuclear foci; in fetal lung fibroblast MRC-5 cells, the nucleus is consistently stained with ~50–100 small foci that are highly concentrated into

two or three areas of the nucleus (Fig. 1 *d* and *inset*). CS22F cells that are equivalent to the MRC-5 cells, but are from adult tissue, also show a similar staining pattern (Fig. 1 *e*). Finally, we tested the staining pattern of RING1 in frozen human foreskin tissue sections, which showed concentrated RING1 foci in keratinocyte nuclei (Fig. 1 *f*) showing that PcG-containing foci can exist in normal cells in situ, as well as in primary-derived cell lines. In these frozen tissue sections we saw no observable difference between the RING1-staining pattern of undifferentiated cells near the basal lamina to that of fully differentiated cells underlying the dead keratinized layer of squames (data not shown). Since in all the cell lines that we studied,
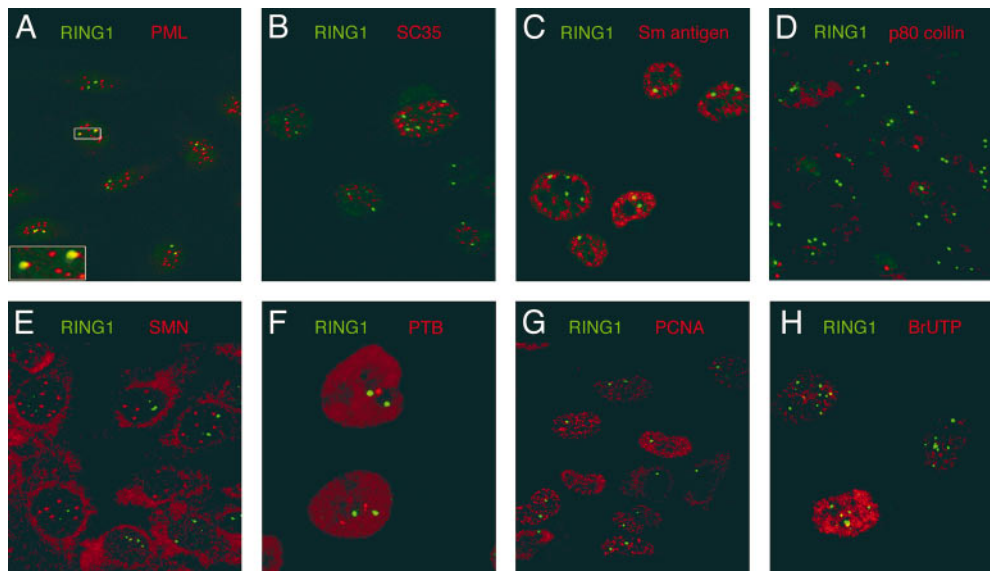
bright concentrated foci are observed when immuno-stained for RING1, BMI1, and hPc2, we thus refer to these nuclear domains as PcG bodies.

PcG Bodies Form a New Class of Nuclear Structure

To test whether PcG bodies are associated with any of the other macromolecular nuclear domains (for review see Lamond and Earnshaw, 1998), we performed rigorous double-immunofluorescent labeling with antibodies against other known and previously characterized nuclear antigens. All double labeling was performed on 2C4 and U-2 OS cells since both contain differing numbers of PcG bodies (see above). For brevity, the data for 2C4 cells is shown in Fig. 2, although both cell lines gave consistent results.

We previously reported that PcG bodies do not colocalize with PML nuclear bodies (Satijn et al., 1997*b*). However, we were able to see an association of some PcG and PML nuclear bodies in single 1-µm optical sections (Fig. 2 *A*; see enlarged area). The significance of this observation is unclear since the association could only be found in ~5% of observed cells, and even more infrequently in U-2 OS cells (data not shown). It is possible that the observed association is due to the 10–20 PML nuclear bodies per nucleus, giving a large probability of random PcG-PML associations. However, on the same rationale one might expect more PcG-PML nuclear body associations in U-2 OS cells since there are between 6 and 14 PcG bodies per nucleus, which is not the case (data not shown). Therefore, the significance of any PcG-PML association will have to await further studies. We also showed previously that RING1 does not colocalize with nuclear domains highly enriched in splicing factors or speckles (Satijn et al., 1997*b*), which is confirmed in the two cell lines used in this study (Fig. 2 *B*).

Here we extend this colabeling study, and show that there is no observable association between PcG bodies and snRNP proteins (Fig. 2 *C*; Lerner et al., 1981), coiled bodies (Fig. 2 *D*; Lamond, 1993), or gemini bodies (Fig. 2 *E*; Liu and Dreyfuss, 1996). The staining pattern observed in mammalian cells for the PTB (Huang et al., 1997) is strikingly similar to that observed for PcG bodies in 2C4 cells; however, we see no colocalization (Fig. 2 *F*). The remaining well-characterized nuclear domains are those

present at sites of DNA replication and RNA synthesis. Sites of DNA replication were visualized using antibodies to the PCNA, but were not found associated with PcG bodies (Fig. 2 *G*). However, the significance of any small degree of association is difficult to interpret because of the large numbers of observed replication foci (Fig. 2 *G*). Similarly, large numbers of RNA synthesis sites can be observed by incorporating the nucleotide analogue bromo-UTP (BrUTP) into nascent RNA and visualizing using an anti-BrUTP antibody. We rarely found PcG bodies localizing with nascent RNA (Fig. 2 *H*), although in highly transcriptionally active cells, some association can be seen (see Fig. 2 *H*). In general we find that PcG bodies as observed in two different cell lines do not significantly colocalize with other known nuclear domains or antigens. However, we do see a clear association between PcG bodies and kinetochores.

PcG Bodies Appear to be Associated with Kinetochores in a Number of Cell Lines

We previously reported that PcG bodies do not colocalize with kinetochore complexes, present at centromeres on all human chromosomes, in human SW480 cells (Satijn et al., 1997*b*). However, upon closer reexamination of numerous labeled cells from different cell lines, we noticed that there was a distinct relationship between the PcG structures and kinetochores revealed by a partial overlap of the two fluorescent signals (see Fig. 3 and Satijn et al., 1997*b*). By analyzing fluorescently labeled PcG bodies and kinetochores on single 1-µm confocal sections, we observe that a significant proportion of both PcG bodies in 2C4 cells always colocalizes with two kinetochore-labeled centromeres (Fig. 3 *a* and *inset*).

Similarly, in U-2 OS cells containing multiple PcG bodies, we observe that all the prominent RING1-labeled PcG bodies give some degree of fluorescent colocalization with kinetochores (Fig. 3 *b* and *inset*). However, one or two of the smallest PcG bodies appear distinct from kinetochores (see Fig. 3 *b*). To test whether this observation is a consequence of cellular transformation, we double-labeled PcG bodies and kinetochores in normal nontransformed primary adult fibroblasts (CS22F cells) where RING1-labeled
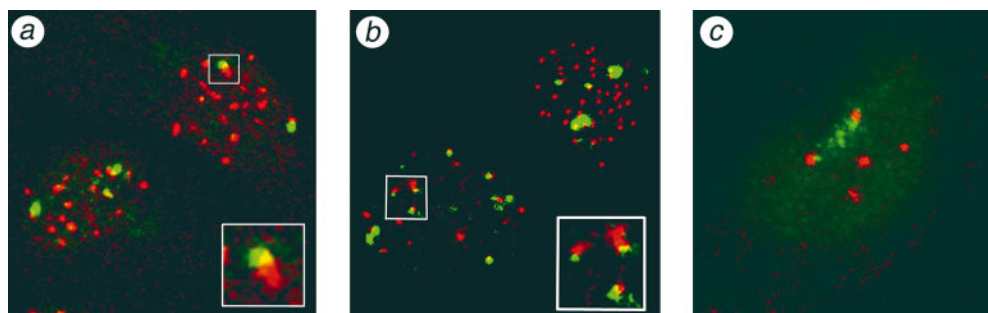
Figure 3. PcG bodies are associated with kinetochores in three different cell lines. Human centromeres were localized using an autoimmune sera against kinetochores, and are shown in red. Their spatial relationship with PcG bodies, labeled with the anti-RING1 antibody (*green*), are shown in (*a*) 2C4 cells, (*b*) U-2 OS cells, and (*c*) CS22F cells. All images are a digital overlay of the two optical channels obtained from a single 1-µm optical confocal section. Any colocalization observed is shown by a yellow signal.

PcG bodies appear as large distinct clusters comprising much smaller foci (see Fig. 1 *e*). A 1-μm section of a CS22F nucleus is sufficient to encompass one such cluster of small PcG bodies (see Fig. 3 *c*, *green*), and double-labeling with the kinetochore antibodies shows that the cluster is associated with one or two centromeres (Fig. 3 *c*).

PcG Bodies Preferentially Localize to Chromosome 1 Territories in 2C4 Cells

The PcG complex in *Drosophila* is known to associate directly with specific chromosomal sites along polytene chromosomes (Zink and Paro, 1989). To study possible chromosomal associations of the human PcG complex, we used a protocol whereby the procedures for immunofluorescent localization of protein complexes and FISH of specific genetic loci were combined to produce a method that allows the identification of any PcG body–chromatin association in interphase cells. By labeling PcG bodies with the anti-RING1 antibody and sequentially hybridizing chromosome-specific paints to interphase 2C4 nuclei, we find that PcG bodies present in these cells consistently localize to regions of the nucleus occupied by chromosome 1 (Fig. 4). We do not see any instance where PcG bodies are not localized with chromosome 1, nor do we see any colocalization or association with any of the other chromosome territories (data not shown). This approach provides us with evidence that PcG bodies in 2C4 cells may be chromosomally associated, as is the case for the PcG complex in *Drosophila*. We therefore used this technique to map the association of PcG bodies finely with chromosome 1 and related sequences in a variety of cell lines.

PcG Bodies Associate with Pericentromeric Heterochromatin in Three Different Cell Lines

Using the protein–chromatin immuno-FISH labeling method described above, but reducing the size of the hybridizing probe, we can specifically localize PcG bodies to areas of constitutive heterochromatin in the pericentromeric region of chromosome 1. The DNA probe pUC1.77, which is specific for the pericentromeric region q12 of chromosome 1 (Cooke, 1979), gives total immunofluorescent colocalization with PcG bodies identified using the anti-RING1 antibody (Fig. 5 *A* [*i*]). This colocalization is identical for both pairs of chromosomes in all interphase cells showing the specificity of PcG bodies for this region of chromosome 1 in 2C4 cells. Furthermore, discrete areas of hybridization of the pUC1.77 probe overlap with PcG body staining in chromatin released from a nucleus using a cytospin (Fig. 5 *A* [*ii*]). This overlap suggests that RING1, as observed in the large foci in 2C4 interphase cells, is concentrated into smaller foci spanning almost the entire q12-hybridizing region of chromosome 1 (see Fig. 5 *A* [*ii*]; *arrowheads*). Given the demonstrated biochemical associations among RING1, BMI1, and hPc2 (Satijn et al., 1997*b*), it would seem likely that all three components are present in these smaller RING1 foci.

Since only two PcG bodies are observed in 2C4 cells, we were interested in studying other cell lines that contain numerous PcG bodies, since large numbers of PcG bodies cannot all localize to the 1q12 region. In U-2 OS cells, the pUC1.77 probe used consistently hybridizes to three major
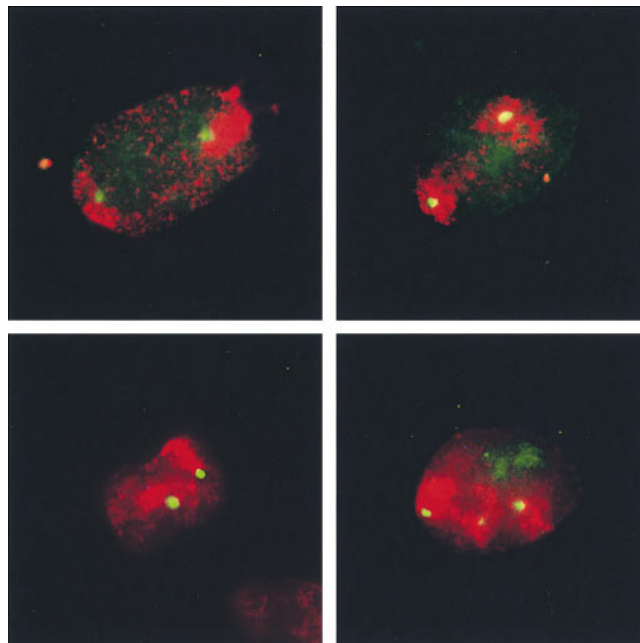

Figure 4. PcG bodies preferentially associate to chromosome 1 territories in 2C4 cells. Digitally merged images of numerous interphase 2C4 cells localizing the chromosome 1 in situ–hybridized probe by immunofluorescence showing the entire chromosome 1 territories (*red*) and immunofluorescent localization of PcG bodies using the anti-RING1 antibody (*green*) are shown. The green channel–labeled PcG bodies can always be seen associated with the red channel–labeled chromosome 1 territories in nuclei of 2C4 cells (*yellow*).

regions of chromatin (Fig. 5 *B* [*i*]; *red*). These three pUC1.77-hybridizing areas were always found concomitant with three of the largest RING1-stained PcG bodies in these cells (Fig. 5 *B* [*i*]; *yellow*). However, as expected a number of smaller PcG bodies remained that were not associated with the 1q12 region. To address this problem, the immuno-FISH experiment on the U-2 OS cells was repeated using a lower hybridization stringency, allowing the pUC1.77 probe to hybridize to any related sequences found in the genome. Using this technique, the pUC1.77 probe now hybridized, albeit with lower affinity, to an additional three sites (Fig. 5 *B* [*ii*]; *arrowheads*). Surprisingly, these lower stringency hybridizing regions consistently colocalized with an additional three or four PcG bodies (Fig. 5 *B* [*ii*]; *arrowheads*). However, a small number of PcG bodies (one or two on average) remained that colocalized neither with 1q12 nor with sequences related to 1q12, as revealed by low-stringency hybridization of the pUC1.77 probe (see Fig. 5 *B* [*ii*]). Nonetheless, these results suggest that in multiple PcG body–containing U-2 OS cells, PcG bodies associate with the 1q12 region, and to chromatin regions related to 1q12. To explain why three major pUC1.77-specific signals were detected in U-2 OS cells, metaphase chromosomes were prepared and hybridized with the pUC1.77 probe. This hybridization revealed that one of the chromosome 1 homologues in this cell line has been rearranged with the breakage point within the pericentromeric heterochromatin recognized by the pUC1.77 probe (Fig. 5 *B* [*iii*]; *double white arrowheads*), thus giving
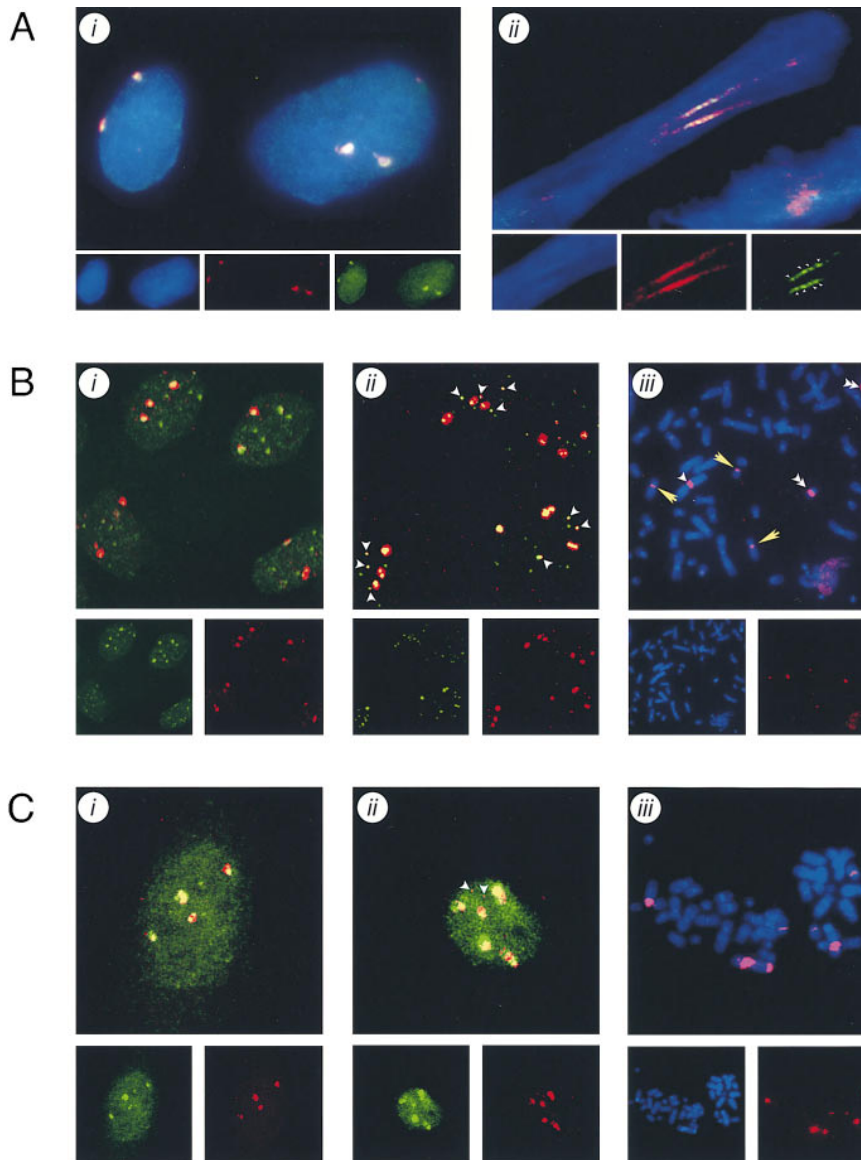
Figure 5. PcG bodies localize specifically to the pericentromeric region of chromosome 1 in a number of cell lines. Digitally merged fluorescent-channel images are shown of interphase cell nuclei showing immunofluorescent localization of the in situ–hybridized pUC1.77 probe, specific for the q12 pericentromeric region of chromosome 1 (*red*) and immunofluorescent localization of PcG bodies using anti-RING1 antibodies (*green*). Nuclei were counterstained with DAPI (*blue channel*) showing the nuclear volume. The separate color channels are shown under the main merged images. (*A*) Interphase 2C4 cell nuclei showing (*i*) normal interphase nuclei and (*ii*) interphase chromatin from a released nucleus by a cytospin where the nuclear architecture and territories are compromised, thereby releasing the chromosome domains throughout the length of the nucleus. (*B*) U-2 OS cells labeled after (*i*) the normal high-stringency immuno-FISH protocol, and (*ii*) lower stringency immuno-FISH protocol showing additional regions of pUC1.77 hybridization that colocalize with smaller PcG bodies (*arrowheads*); (*iii*) low-stringency hybridizations of the pUC1.77 probe were localized on metaphase chromosome spreads counterstained with DAPI (*blue channel*) showing the three large 1q12-specific hybridizing regions (*white arrowheads*) that are due to breakage of the q12 region in one chromosome 1 (*double white arrowheads*), together with the additional sites of hybridization during low-stringency conditions (*yellow arrowheads*). (*C*) SAOS-2 cells labeled after hybrdization of the pUC1.77 probe (*i*) and hybridization at lower stringency showing an additional two sites of hybridization (*ii*; *arrowheads*); (*iii*) low-stringency hybridizations of the pUC1.77 probe were localized on metaphase chromosome spreads counterstained with DAPI (*blue channel*), showing the multiple rearrangements of the 1q12 region.

three prominent signals per cell (Fig. 5 *B* [*iii*]; *white arrowheads*). The remaining genomic regions recognized by the pUC1.77 probe at lower stringency hybridizations were also found to be pericentromeric on other, as yet unidentified chromosomes (Fig. 5 *B* [*iii*]; *yellow arrowheads*).

In SAOS-2 cells the pUC1.77 probe hybridizes to four regions of chromatin (Fig. 5 *C* [*i*]; *red channel*). An analysis of metaphase chromosomes from SAOS-2 cells demonstrated that this was again due to rearrangements involving chromosome band 1q12 (see Fig. 5 *C* [*iii*]). These pUC1.77-hybridizing regions were identified as rearranged 1q12 areas by hybridizing chromosome 1–specific probes to these metaphase spreads (J. Williamson, unpublished observations). Low-stringency hybridization of the pUC1.77 probe gave an additional two hybridizing regions that did not colocalize to PcG bodies (Fig. 5 *C* [*ii*]; *arrowheads*).

However, in this cell it is demonstrated that all the large PcG bodies are once again concurrent with the larger areas of pUC1.77 hybridization (see Fig. 5 *C*; *yellow*), demonstrating that the specific association of PcG bodies to the 1q12 region is not restricted to one cell type.

PcG Bodies Remain Associated to Pericentromeric Chromatin Regions Throughout Mitosis

To study the dynamics and stability of PcG bodies during mitosis and cell division, we fixed and labeled cells with propidium iodide to identify mitotic cells. It was previously observed by others that the large nuclear mammalian PcG protein–containing complexes were not antibody-labeled during cell division until the latter stages of mitosis. However, we attribute this finding to the possible

destruction of protein antigenicity by fixation (see Materials and Methods). Using a milder fixation protocol while maintaining attachment of mitotic cells to the coverslip, we are able to use double immunofluorescent labeling with propidium iodide and FITC-labeled PcG components (RING1, BMI1, and hPc2) to study PcG bodies through all stages of mitosis in 2C4 cells (Fig. 6 A) and RING1 in U-2 OS cells (Fig. 6 B). Upon chromosome condensation at prophase, PcG bodies become much reduced in size and less conspicuous than those in interphase cells (Fig. 6). However, upon disassembly of the nuclear envelope and matrix at prometaphase, PcG bodies are once again, large concentrated foci seen by staining with either the anti-hPc2 or anti-BMI1 antibodies (Fig. 6 A). Interestingly, in the majority of cells at this stage, PcG bodies have doubled in number (see Fig. 6 A). This is most apparent in one particular 2C4 cell, which contains four PcG bodies, probably

due to the known small number of tetraploid cells present in this cell line (J. Williamson, unpublished observations). Here we are able to see eight PcG-stained regions that associate on both pairs of chromatids concentrated into smaller foci (Fig. 6 I; arrowheads). This is similar to the pattern observed in the chromatin-released cell with RING1 associating to 1q12 in discrete smaller foci (see Fig. 5 A [ii]; arrowheads). We are unable to locate RING1 in 2C4 cells at prometaphase (Fig. 6 A), although we can see RING1 at a similar stage in U2-OS cells (Fig. 6 B). This is surprising, since we observe RING1 when the chromosomes align at the metaphase plate during metaphase (Fig. 6). BMI1 and hPc2 can also be clearly seen as part of the duplicated PcG bodies during metaphase (Fig. 6 A). We attribute this observation either to loss of RING1 from PcG bodies, or more likely to an inaccessibility of RING1 to antibody labeling.
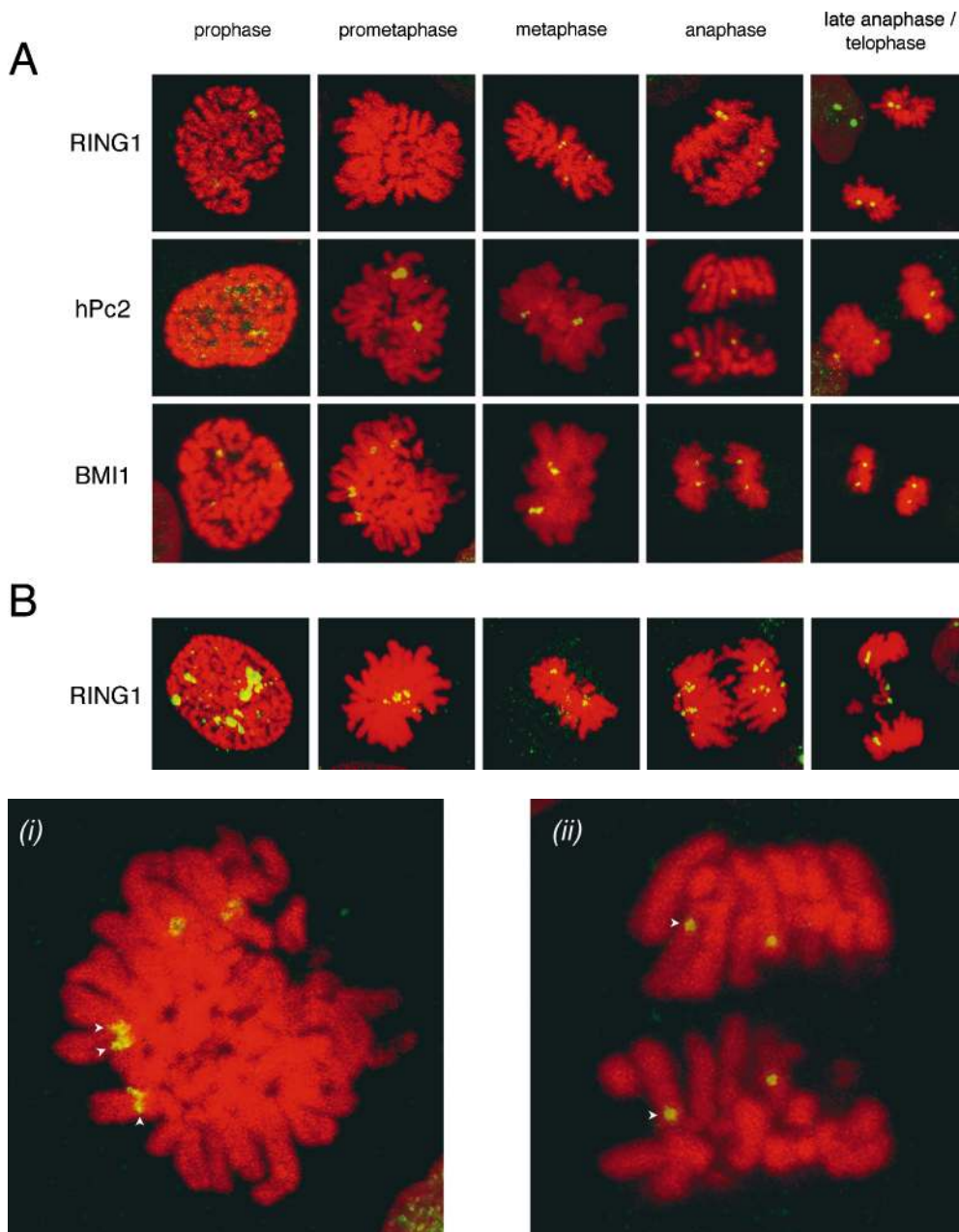

Figure 6. PcG bodies remain chromosomally associated throughout mitosis. Mitotic chromosomes were visualized by propidium iodide staining of formaldehyde-fixed cells, and are shown in red. The various stages of mitosis were distinguished according to criteria used by Chaly et al. (1984). PcG bodies were visualized by immunofluorescent labeling of (*A*) 2C4 cells with antibodies against either RING1 (*top*; *green channel*), hPc2 (*middle*; *green channel*) or BMI1 (*bottom*; *green channel*) and (*B*) U-2 OS cells with antibodies against RING1 (*green channel*). A digital overlay of the two-color channels shows the relation of PcG bodies with the condensed chromosomes during the given stages of mitosis. (*i*) Enlargement of BMI1 during prometaphase in 2C4 cells in the main figure shows direct chromosome association with concentration of the protein on the sister chromatids (*arrowheads*). (*ii*) Enlargement of hPc2 during anaphase in 2C4 cells in the main figure shows direct chromosome association of hPc2 to a pericentromeric region on chromosome 1 (*arrowheads*).

At anaphase, the PcG bodies present during prometaphase and metaphase are evenly segregated to each set of separating chromatids (Fig. 6). In one particular 2C4 anaphase cell, we can visualize a pericentromeric association of a PcG body with a larger chromosome, which, based on our FISH experiments is likely to be chromosome 1 (Fig. 6 *ii*, *arrowheads*). This would suggest that, at least in 2C4 cells, PcG bodies maintain their association with the pericentromeric region of chromosome 1 through to the daughter nuclei. As the daughter cells form in late anaphase/telophase, PcG bodies are more clearly seen, and are similar in size to those present in interphase cells (Fig. 6). In summary, by labeling PcG bodies with antibodies to RING1, BMI1, and hPc2, in two human cell lines we observe that (*a*) the core structural integrity of PcG bodies is maintained throughout mitosis; (*b*) the structures are found closely associated with condensed chromosomes; and (*c*) their association with chromosomes is maintained by duplicating PcG bodies with subsequent segregation of the PcG complex as bound to both sets of separating chromatids, to the newly formed daughter cells.

Discussion

Human PcG Components Localize to Novel Nuclear Foci in a Number of Cell Lines

The recent identification of multiprotein complexes containing a number of mammalian PcG homologues in nuclei of human cells (Alkema et al., 1997; Gunster et al., 1997; Satijn et al., 1997*b*) has raised interesting questions as to how the mammalian PcG homologues and complex function. Through immunoprecipitation experiments and yeast two-hybrid analyses, it has recently been shown that several mammalian PcG proteins including BMI1 (*Xenopus*, mouse and human), RING1 (mouse and human), Pc (*Xenopus* and mouse), hPc2, MPh/Rae28, HPH1, and HPH2 interact in vivo and localize to discrete nuclear multiprotein complexes (Alkema et al., 1997; Gunster et al., 1997; Hashimoto et al., 1998; Reijnen et al., 1995; Satijn et al., 1997*b*; Schoorlemmer et al., 1997). Furthermore, RING1 and hPc2 are able to repress gene activity when targeted to a reporter gene, further supporting their proposed functional role as part of a human PcG complex (Satijn et al., 1997*b*; Schoorlemmer et al., 1997). Mouse knockout and transgenic experiments also suggest that several PcG-related genes have direct roles in embryonic development analogous to the PcG complex in *Drosophila* (for review see Gould, 1997), although the molecular mechanisms and functions for the mammalian PcG complex still remain unknown.

Our previous results (Satijn et al., 1997*b*) and those presented here clearly show that the three human PcG proteins—RING1, BMI1 and hPc2—colocalize to discrete nuclear foci or bodies in a variety of cell lines that we term PcG bodies. Moreover, PcG bodies also exist in nontransformed primary cell lines including human fetal and adult fibroblasts and keratinocytes from human tissue sections, suggesting that they are not a consequence per se of cellular transformation and subsequent overexpression. However, PcG bodies do vary both in number and size from the very distinct two large PcG bodies seen in the fibrosarcoma 2C4 cell nuclei to the much smaller and more numerous PcG bodies observed in normal human fibroblasts, which may reflect different expression levels or chromosomal rearrangements (see below). It is interesting to note that in both fetal (MRC-5) and adult (CS22F) fibroblasts, the small PcG bodies are not randomly situated within the nucleus, yet appear to cluster into defined areas of the nucleus (see Fig. 1, *d* and *e*), suggesting that at least in these cells, PcG bodies are forming higher-order structural arrangements that may be of functional importance. However, we are unable at present to determine the specificity for size and number of PcG bodies per cell, nor what functionality exists for such diversity. It is notable though, that in fixed whole-mount developing *Drosophila* embryos, a varied nuclear distribution of three PcG proteins is observed, giving rise to approximately 100 nuclear foci of differing sizes (Buchenau et al., 1998). These data show that PcG bodies in a developing organism are inherently variable in numbers and morphology. Indeed, some of the PcG foci observed by Buchenau et al. (1998) are of comparable size (as estimated by indirect immunofluorescence) to those we observe in tissue culture cells, although at present the functional significance of such a varied PcG distribution is not known (Buchenau et al., 1998).

Since there is a large number of well-characterized and distinct nuclear multiprotein complexes or bodies, it is important to define human PcG bodies in relation to other known nuclear complexes. We find that PcG bodies do not coimmunolocalize with any other known nuclear body, although we do observe some association, albeit infrequently, between PcG bodies and PML nuclear bodies. PML nuclear bodies have previously been observed to be associated closely with DNA replication domains in middle to late S-phase, and it has been suggested that PML bodies are associated with specific genomic loci (Grande et al., 1996), which may explain our observations. At present we do not know the functional significance of the PML–PcG association. We do observe, however, a significant association between PcG bodies and centromeres as visualized with the human autoimmune serum against kinetochores, H33 (described in Stuurman et al., 1992). The PcG body signals obtained with the anti-RING1 and anti-kinetochore antibodies were partially overlapping in 2C4, U-2 OS, and CS22F cells (see Fig. 3) as well as in SW480 cells (Satijn et al., 1997*b*), indicating that although not directly colocalizing with centromeres, PcG bodies localize to regions very close to some centromeres. An association of PcG bodies close to human centromeres suggests a chromosomal association for PcG bodies that we further investigated using a protocol termed immuno-FISH. This technique combines immunofluorescent labeling of PcG bodies and FISH of specific human chromosome probes. While this manuscript was in preparation, a similar modified method was also described elsewhere (Brown et al., 1997).

PcG Bodies Associate with Pericentromeric Heterochromatin in Cultured Cells

Using the immuno-FISH technique to systematically label individual chromosome territories while colabeling PcG bodies with the anti-RING1 antibody, we showed a prefer-

ential localization of PcG bodies to chromosome 1 territories. This gave the first preliminary evidence that the human PcG complex associates directly with chromatin. We used smaller probes against specific regions of chromosome 1 to specifically identify the region of the chromosome with which PcG bodies associate. We find direct PcG complex–chromatin association with the q12 region of chromosome 1 in three different cell lines. In 2C4 cells, RING1 associates with the entire 1q12 region, concentrating in smaller domains throughout the pUC1.77-hybridizing region (see Fig. 5 A [ii]). It is likely that other PcG proteins will be found at these smaller RING1 sites given the known biochemical interactions of RING1 with BMI1 and hPc2 (Satijn et al., 1997b). In cell lines that contain numerous PcG bodies, in addition to binding to 1q12 we demonstrated that the PcG bodies show an affinity for chromatin regions whose sequences are related to 1q12. Performing the immuno-FISH protocol using a lower stringency for hybridizing the pUC1.77 probe, we observed three additional pUC1.77-hybridizing regions that lie pericentromeric on other chromosomes, and also contain associated PcG bodies (see Fig. 5 B [ii and iii]). It is noteworthy that in the U-2 OS cell line, the PcG bodies that are associated with 1q12-related sequences are considerably smaller in size than the largest PcG bodies that are directly associated with 1q12 (compare Fig. 5 B [ii and i]). This result could suggest that primarily, the PcG bodies associate with 1q12, but once these sites are saturated (for example because of an overproduction of the PcG complex components), the PcG proteins form additional PcG bodies associating with chromatin-containing 1q12-related sequences. However, this is highly suggestive of the human PcG complex demonstrating specificity for a DNA sequence, and the scope of this study cannot discern whether the PcG bodies directly associates with the 1q12 (and related) sequence or whether it is associated through protein–protein interactions with DNA-binding proteins already present at these sites. Nonetheless, all the PcG bodies in the 2C4, U-2 OS, and SAOS-2–transformed cell lines, as well as those in the normal primary CS22F cells, clearly show a preference for chromatin surrounding some human centromeres (Figs. 3 and 5). Unfortunately, we were unable to perform the immuno-FISH protocol on the normal MRC-5 and CS22F cells to determine whether the large clusters of PcG domains (see Fig. 1, d and e) correspond to regions of pUC1.77 hybridization. This result was due to loss of protein immunofluorescence signal during the hybridization stage of the FISH protocol (data not shown). Thus, more refinement of the immuno-FISH method is needed in order to preserve smaller protein signals before this can be determined.

The pericentromeric q12 region of chromosome 1 is highly heterochromatic, comprising the largest amount of satellite 2 sequence in the human genome, and a minor amount of satellite 3 (Tagarro et al., 1994), with the pUC1.77 probe hybridizing to the satellite 2 sequence (Cooke, 1979; Lee et al., 1997). Satellites 2 and 3 are related sequences of satellite DNA comprising primarily a repeated ATTCC pentameric sequence (Prosser et al., 1986). However, satellite 2 sequences are poorly conserved (reviewed in Lee et al., 1997); the satellite 2 sequence on chromosome 1 that constitutes the pUC1.77

probe is a unique partition of the family (Cooke, 1979), and only shows hybridization to related sequences under low-stringency conditions (see Fig. 5 B [iii]). In the three cell lines studied, we found PcG bodies associated to pUC1.77-hybridizing chromatin, even at the additional pericentromeric sites located during low-stringency hybridizations. The size of the 1q12 region is known to be highly variable, and thus it is possible that in the large PcG body–containing cells, this region has expanded, thereby allowing accumulation of large amounts of PcG proteins (data not determined). This affords a possible explanation as to the large size of the two 1q12-associated PcG bodies found in the 2C4 cell line, resulting in depletion of PcG bodies at 1q12-related sequences found in other cells.

PcG Bodies are Stably Associated with Chromatin Through Mitosis

We demonstrate that three different human PcG components (RING1, BMI1 and hPc2) remain chromatin-associated near centromeric regions throughout all stages of mitosis, and that around the prometaphase/metaphase stage of the mitotic cycle, the PcG complex appears to redistribute so that each chromatid contains bound PcG complex. We also observe a similar PcG complex-mitotic chromatin association in U2-OS cells that contained numerous PcG bodies, suggesting that the maintenance of chromosome-bound PcG complexes during mitosis is not limited to 2C4 cells. Furthermore, murine Pc (M33) and Bmi-1 have also been localized to metaphase chromosomes (Wang et al., 1997). More recently, it was estimated that between 5 and 7% of three PcG proteins (PC, PSC and PH) are bound to metaphase chromosomes in developing *Drosophila* embryos (Buchenau et al., 1998). One interpretation of these observations is that by maintaining chromosome-associated PcG complexes during mitosis, the proteins constituting PcG bodies are ensured to be inherited by successive cell generations, thus providing the daughter cells with the necessary components for maintaining gene expression patterns. Although this is speculative, it will be important to determine whether the association of PcG complexes with mitotic chromosomes is an important feature of overall PcG function.

What are the Functional Implications of Human PcG–Heterochromatin Association?

The observation that the human PcG complex associates directly with pericentromeric heterochromatin in a number of different cultured cell lines is intriguing and suggestive. Here we provide direct evidence for mammalian PcG association with specific and related DNA sequences found pericentromeric on a number of different chromosomes in three different human cell lines, as well as an association close to centromeres in one normal primary cell line. Support for these observations comes from other studies, including the report of murine Pc homologue M33 bound to pericentromeric regions on metaphase chromosomes (Wang et al., 1997), and also from more recent data on PcG protein distribution in developing *Drosophila* embryos (Buchenau et al., 1998). In the study by Buchenau and coworkers, one PcG protein PSC is often localized as a cluster of spots that are very near to or partially overlap-

ping with centromeric heterochromatin in interphase nuclei (Buchenau et al., 1998). These observations are similar to those reported here for RING1 staining in primary cultured embryonic fibroblasts, showing RING1 clustered as small dots in the proximity of kinetochores (see Fig. 3 *c*). Given that the observations for PSC are in full *Drosophila* embryos and not cultured cells, there is now direct evidence for at least one PcG component associating with centromeric heterochromatin in interphase nuclei in a developing organism. However, what determines the specificity for PcG proteins at centromeric regions in *Drosophila* (Buchenau et al., 1998) and mouse cells (Wang et al., 1997) is not known since satellite 2 sequences do not exist in these organisms.

What would be the functional significance for such an association? There are a number of possible interpretations, none of which have any direct experimental evidence to date, and all are thus purely speculative.

Firstly, the PcG bodies described in this study could represent storage domains where surplus PcG proteins are stored until required by the cell. Indeed, if PcG bodies are used in this way, then the fidelity of segregation of the complex during mitosis represents a way for the cell to redistribute evenly essential repression components required for immediate transcriptional repression after cell division. Recent studies on GAGA factor, a known transcriptional activator that counteracts chromatin-induced repression and is responsible for heterochromatin decondensation (Farkas et al., 1994), are of interest in this context. GAGA factor and trithorax (trx) have been shown to colocalize with Pc at PcG/trxG-regulated regions on *Drosophila* chromosomes (Chinwalla et al., 1995; Strutt et al., 1997). GAGA factor has also been shown to localize to areas of heterochromatin in centromeric regions of chromosomes from *Drosophila* embryos and cells in culture (Raff et al., 1994). More surprisingly, however, is the observation that GAGA factor remains associated to centromeric regions during mitosis (Raff et al., 1994), but appears to be relocalized to euchromatic binding sites after cell division (Platero et al., 1998). A similar redistribution from heterochromatin to euchromatin has also been observed for the *Drosophila* gene regulatory protein PROD (Platero et al., 1998; Török et al., 1997). Thus, GAGA factor and PROD appear to have some cytological similarity with the human PcG complex reported here in that they bind heterochromatin during mitosis.

Secondly, could pericentromeric heterochromatin-associated PcG complexes play an active role in transcriptional silencing? The powerful transcriptional silencing effect that constitutive heterochromatin imposes on juxtapositioned euchromatic genes is well-documented. The effect, known as position-effect variegation (PEV), gives rise to unstable gene expression as a result of positioning euchromatin into heterochromatin regions (for review see Reuter and Spierer, 1992), although it is not known whether constitutive heterochromatin regions and PEV constitutes an active role in gene regulation (for example, see Henikoff, 1995) or directly relates to PcG-mediated gene silencing (McCall and Bender, 1996). However, recent data has shown that during human B cell development, genes are rendered transcriptionally inactive by selectively targeting that gene to centromeric heterochromatin regions (Brown

et al., 1997), providing direct evidence that centromeric heterochromatin plays an active role in transcriptional regulation in interphase cells. The main experimental evidence against such a mechanism for PcG-mediated gene repression is the observation that multiple PcG foci appear randomly distributed in interphase nuclei, and are highly variable in size and number in both cultured and primary derived cells. More compelling evidence against PcG heterochromatin clustering in vivo comes form the recent work of Buchenau et al., where the spatial distribution of three PcG components has been studied in whole developing *Drosophila* embryos, and reveals no apparent spatial organization (Buchenau et al., 1998). In general, therefore, there is little evidence for a specific nuclear clustering and/or distribution for PcG complexes in interphase nuclei, although there are a few examples of specific single PcG components clustering; PSC in *Drosophila* (Buchenau et al., 1998) and RING1 in MRC5 and CS22F primary cells (this study), the relevance of which is at present unclear.

In summary, we have shown that the human PcG complex forms a novel class of nuclear domain that can localize to regions of constitutive heterochromatin. We have observed specific PcG associations at centromeres on different chromosomes in three human cell lines. Furthermore, PcG complexes associate with repetitive DNA sequences that are related to the satellite 2 band on chromsome 1, providing the first suggestive data that mammalian PcG complexes may be chromatin-associated through a specific DNA sequence found at the centromeric region on a number of chromosomes. Although this result provides no direct evidence for constitutive heterochromatin playing a role in mammalian PcG-mediated transcriptional repression, it establishes for the first time a link between heterochromatin and the human PcG complex, and suggests a variety of experimental approaches and model systems for further investigation into the molecular role of the mammalian PcG complex.

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