

Review

The Role of Polycomb Group Proteins in Cell Cycle Regulation during Development

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KEY WORDS

Polycomb group, chromatin, epigenetics, cell cycle, development.

ABBREVIATIONS

PcG Polycomb Group
PRC Polycomb repressive complex
trxG trithorax Group
PRE PcG response element

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ABSTRACT

Polycomb group (PcG) and trithorax group (trxG) proteins are evolutionarily conserved chromatin modifiers that have well known roles in the maintenance of silent and active expression states of homeotic genes. PcG proteins may also be involved in the control of cellular proliferation, as several PcG complexes have been shown to act either as proto-oncogenes or as tumor suppressors in vertebrates. In *Drosophila*, PcG factors associate with specific DNA regions termed PcG response elements (PREs), and a PRE was recently identified in the gene encoding Cyclin A. Still, it is not yet clear how PcG complexes could control cell cycle progression. Beyond acting as stable silencers of cell cycle genes during the differentiation process, PcG complexes might also be integrators and/or modulators of cell cycle checkpoints in dividing cells. Here, we discuss this dual aspect of PcG involvement in epigenetic cell cycle control.

INTRODUCTION

Polycomb group (PcG)-dependent epigenetic regulation has emerged within the past few years as an important player in the control of proliferation during the acquisition of cell identity. In vertebrates, PcG complexes regulate the self-renewal of various types of embryonic and adult stem cells, and have been implicated in several types of cancer.^{1,2}

The PcG proteins were first discovered as regulators of homeotic (Hox) gene expression, based on mutant phenotypes involving posterior transformations of body segments in *Drosophila* and skeletal malformations in vertebrates.³⁻⁶ PcG proteins are evolutionarily-conserved chromatin modifiers that can transcriptionally silence their targets through many rounds of cell division during development. Their repressive function can be counteracted by activation involving trxG proteins.⁷ The PcG/trxG system therefore provides a cellular memory mechanism.⁷ At the molecular level, PcG complexes achieve stable silencing by introducing specific modifications onto histone tails.

PcG proteins form at least two classes of multimeric chromatin binding complexes: the Polycomb repressive complex 1 (PRC1), and the PRC2/3/4 complex (also known as the Eed-Ezh2 complex in mammals). While the exact composition of these PcG complexes varies, their core components are conserved.⁸ The different complexes are recruited sequentially to their target genes.⁹ The *Drosophila* PRC2 complex is thought to initiate repression, and includes the proteins Enhancer of zeste (E(z)), Suppressor of zeste 12 (Su(z)12), NURF-55, and Extra sex combs (Esc).¹⁰ PRC2/3/4 complexes can trimethylate Lysine 27 of histone H3 (H3K27me3) in vivo, and Lysine 26 of histone H1 (H1K26me3) in vitro.¹¹ The PRC1 complex, which contains Polycomb (PC), Polyhomeotic (Ph), Posterior Sex Combs (Psc), and Sex combs extra (Sce/dRing),¹² can be recruited by H3K27me3 (see Fig. 1) via binding of the PC protein chromodomain.^{13,14} Once bound to chromatin, the complex can repress transcription by preventing ATP-dependent nucleosome remodeling by the SWI/SNF complex¹⁵ as well as by establishing direct contacts with the transcriptional machinery.¹⁶ It has recently been demonstrated that PRC1 also has a ubiquitin E3 ligase activity that targets lysine 119 of histone H2A and is involved in gene silencing.^{17,18}

Members of the PRC1 and PRC2/3/4 complexes have been implicated in cell proliferation control, for example by acting either as proto-oncogenes or as tumor suppressors in vertebrates. In *Drosophila*, it has long been known that PcG mutations also cause cell cycle defects. For instance, null alleles of *E(z)* display a small disc phenotype with no discernible mitotic figures.¹⁹ Despite these observations, however, it remains unclear how PcG complexes may control cell cycle progression. Here, we propose that PcG complexes might

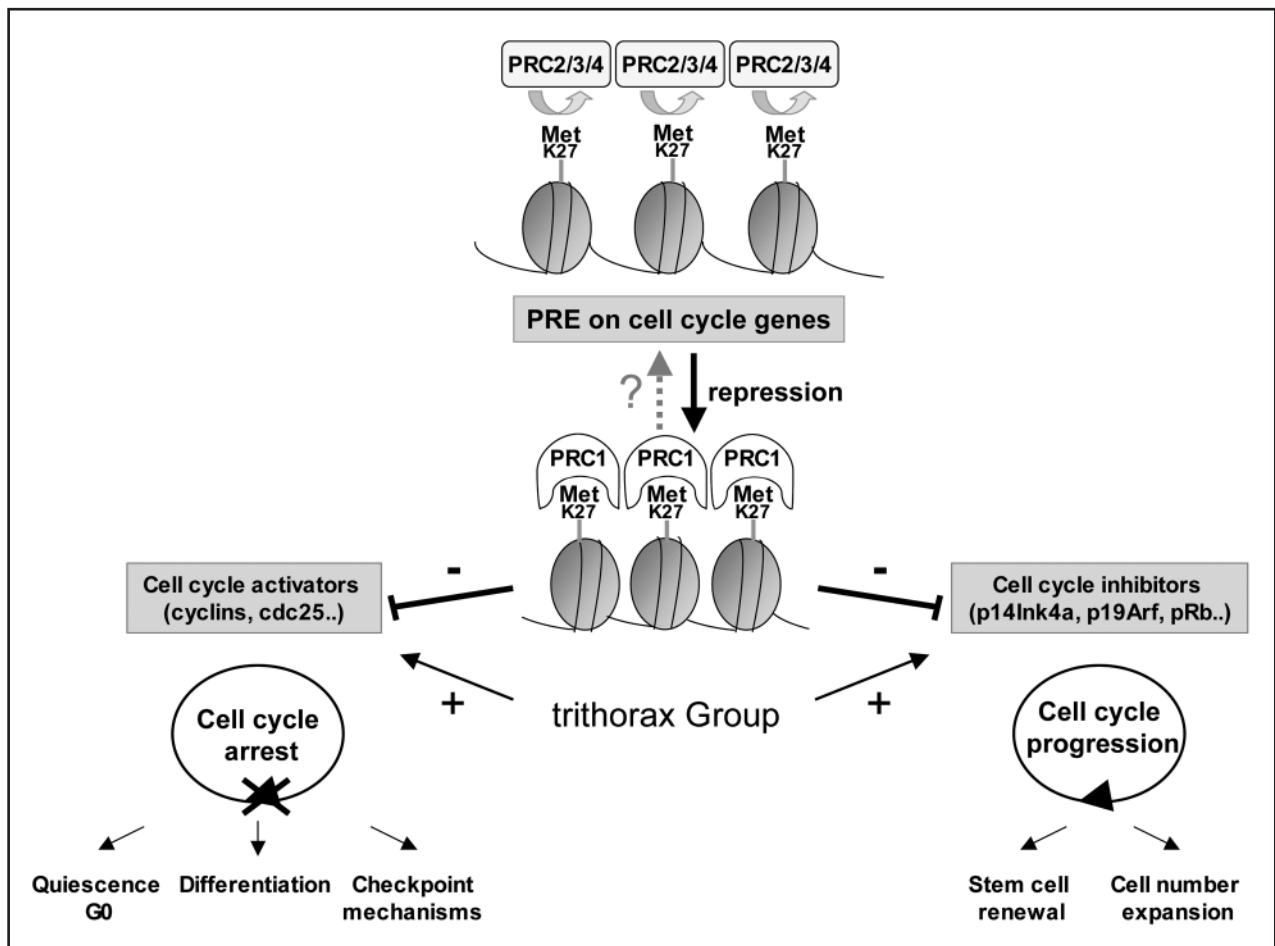


Figure 1. PcG complexes: tumor suppressors versus proto-oncogenes. A hypothetical model is presented illustrating how PcG complexes could behave either as tumor suppressors or as proto-oncogenes. The PRC2/3/4 complexes might label cell cycle genes by repressive marks on their PREs. These marks could then be recognized by PRC1 complexes. This configuration locks cell cycle genes in a transcriptionally repressed state. If the target is an activator of the cell cycle, then proliferation is arrested, whereas if the target is an inhibitor of the cell cycle machinery, then proliferation is activated. Members of the *trxG* might counteract the effects of the PcG complexes on these cell cycle genes.

not behave solely as stable silencers of cell cycle genes during the differentiation process, but also as integrators and/or modulators of cell cycle checkpoints in dividing cells. This dual function might rely on previously neglected, dynamic features of PcG activity that enable PcG proteins to respond to several signaling pathways.

DIRECT OR INDIRECT CONTROL OF THE CELL CYCLE BY PcG COMPLEXES?

PcG members have been proposed to regulate the cell cycle in at least three different ways. First, the cell cycle defects observed in PcG mutants could be an indirect consequence of modifications in Hox gene activities that positively or negatively affect cellular proliferation.^{20,21} In agreement with this possibility, it has been shown that several Hox genes are involved in the control of cell proliferation.^{4,22,23} In mice, for example, *Hoxa1* mutants may have cell growth defects,²⁴ and *Hoxd3* has been suggested to regulate the proliferation rates of precursor cells.²⁵ Also, mice lacking *Hoxd13* products display a limb phenotype that could be the result of a cell proliferation defect.²⁴ In humans, *Hoxb4* expression is involved in hematopoietic stem cell proliferation.²⁶ Finally, the misregulation of some Hox genes leads to alterations in lymphocyte proliferation.^{22,27}

A second possibility is that PcG-mediated gene silencing is linked to general chromatin condensation as cells progress through the cell cycle. Chromatin perturbations, for example, could lead to genome instability and mitotic defects. It has been shown by chromatin immunoprecipitation analysis that the *Drosophila* topoisomerase II and Barren proteins, which are required for proper condensation in mitosis, are present at sequences within the homeotic bithorax complex and at promoters that are specifically targeted by PcG complexes.²⁸ Furthermore, *Drosophila* Ccf, a centrosomal and chromosomal factor that is required for proper condensation of mitotic chromosomes, colocalizes with Psc-binding sites on polytene chromosomes.²⁹ Finally, mutations in *ph*, *Pc* and *Psc* display segregation defects caused by the formation of anaphase bridges during syncytial embryonic mitoses.³⁰ Interestingly, the *ph* locus contains two neighboring transcription units, *ph^p* and *ph^d*, which encode closely related proteins that can partially substitute for each other.³¹ Despite these close similarities, however, only *ph^p*, and not *ph^d*, exhibits segregation defects. The specificity in the cell cycle control of different PcG members can also be seen in the finding that mutations in *E(z)* show condensation and metaphase defects, but do not display anaphase bridges. Taken together, these results point towards a structural function of the different PcG members in controlling mitosis.

Finally, PcG complexes might directly regulate the transcription levels of cell cycle genes. In this regard, work done in vertebrates and invertebrates has highlighted the potential involvement of PcG proteins in the main cell cycle checkpoint pathways. Much work remains to be done regarding this third possible mechanism. Despite extensive research, for example, formal proof of PcG-mediated regulation of vertebrate cell cycle genes via direct binding to regulatory DNA regions is still missing.

PCG COMPLEXES AND CELL CYCLE CONTROL IN VERTEBRATES

Initial evidence supporting the direct control of the cell cycle by PcG proteins in vertebrates came from studies on mouse *Bmi1/Pcgf4* (a homolog of *Drosophila Psc*), which was first identified as a proto-oncogene that cooperates with *c-Myc* to promote the generation of mouse B- and T-lymphomas.^{32,33} More recently, human *BMII/PCGF4* was shown to be directly transcriptionally activated by *c-Myc*, suggesting a possible mechanism for the cooperation between the two genes.³⁴ Loss of function of *Bmi1* has pleiotropic effects. Mice lacking *Bmi1* exhibit a homeotic posterior transformation coupled with strong proliferative defects during lymphocyte development, and also neurological abnormalities.⁶ *Bmi1* was later shown to be necessary for the efficient self-renewal of hematopoietic stem cells as well as neural stem cells in the peripheral and central nervous systems. *Bmi1* has only a relatively minor impact on the differentiated progeny of these cells, however.³⁵⁻³⁷ Finally, in the absence of *Bmi1*, primary embryonic fibroblasts are unable to progress into S phase and to undergo premature senescence.³⁸

The tumor suppressors *p16INK4a* and *p19ARF* have been shown to be downstream targets of *Bmi1* in vivo, as removal of *INK4a/ARF* alleviates the lymphoid and neurological proliferation defects caused by the loss of *Bmi1*.³⁸ It was recently shown that *INK4a* and *ARF* have different cell type specificities with respect to their requirements for *Bmi1*-mediated control of cell proliferation.³⁹ Specifically, *INK4a* is required for *Bmi1*-dependent self-renewal in neural stem cells, and *ARF* has the same property in hematopoietic stem cells. Importantly, however, the *INK4a-ARF* locus cannot account for all aspects of the hematopoietic and neurological defects observed in *Bmi1* null mutants, indicating that additional cell context-dependent targets of *Bmi1*-containing complexes remain to be identified. Further, because *Bmi1* is expressed in multiple tissues, it is possible that additional examples of *Bmi1* regulating cellular proliferation will come to light.⁴⁰ In human breast carcinomas, for instance, *BMII* is able to activate the telomerase reverse transcriptase (hTERT) independently of *c-Myc* binding sequences.⁴¹ It is clear, however, that many other genes are affected by the loss of *Bmi1* in hematopoietic stem cells or neurospheres, including Hox family members and p53-dependent genes.³⁶

The observation that PcG expression levels and progression through the cell cycle are closely aligned in the hematopoietic cell lineage also argues in favor of direct PcG-dependent proliferation control in mammals. It has been shown, for example, that the differentiation of primary bone marrow cells is generally accompanied by an increase in PcG gene expression levels,⁴² with the exception of *BMII*, which is expressed at high levels in progenitor cells and very low levels in mature (differentiated) cells.⁴² In all PcG mutants analyzed so far, the hematopoietic compartment that is most strongly affected corresponds to the gene's preferential expression domain, e.g., stem cells in the case of *BMII* and mature cells with *MEL-18/PCGF2* (a close relative of *BMII* and a second homolog of *Psc*),

M33/CBX2 (a *Pc* homolog), and *HPH1/PHC1* (a *ph* homolog).⁴² In a large number of cancers, a clear correlation has been established between the aberrant expression of mammalian PcG members and tumorigenesis.⁴³⁻⁴⁷

PcG members can target a wide spectrum of cell cycle genes, including inhibitors of cell cycle progression (*INK4a/ARF* locus), cryptic transcriptional regulators (*c-Myc*, *c-Jun*, *c-Fos*), and even activators of the cell cycle machinery (*cdc25*). For instance, overexpression of *RING1* has a proto-oncogenic effect. Although this is also true of *Bmi1*, the overexpression of *RING1* is accompanied by a strong increase in the expression of the proto-oncogenes *c-Jun* and *c-Fos*,⁴⁸ but not, in contrast to *Bmi1*, *c-Myc*.

PcG proteins do not always play positive roles in cell proliferation. For example, while *Bmi1* is a proto-oncogene, its homolog *Mel-18* has been proposed to act as a tumor suppressor,⁴⁹ and human MEL-18 has been found to be downregulated in breast cancer cell lines.⁵⁰ *Mel-18/Pcgf2* inhibits proliferation in differentiated B-lymphocytes by downregulating the *c-Myc/cdc25* pathway, leading to the downregulation of cyclins and CDKs.⁵¹ Recently, *Mel-18* was also proposed to repress hematopoietic stem cell self-renewal and promote their differentiation.⁵² These contradictory results might indicate that the cell cycle effects of *Mel-18* and *Bmi1* are indirect downstream consequences of their functions, or that *Mel-18/Bmi1* can target tumor suppressor genes in some conditions/tissues and proto-oncogenes in others. In particular, these two proteins might compete each other in the formation of PcG protein complexes with opposing functions (see below). Overexpression experiments could clarify this aspect of *Mel-18* function. Interestingly, hPC2/CBX4 (a human homolog of *Pc*) has also been shown to repress the expression of the proto-oncogene *c-Myc*⁵³ and to behave as a tumor suppressor, in this case by acting in collaboration with E2F and pRb. Finally, mouse PRC2 member *Eed* has been shown to antagonize *Bmi1* and have a repressive effect on hematopoietic cell proliferation. Significantly, human EED, as with the other EZH2 members, EZH2 and SUZ12, is regulated by the pRB-E2F pathway and is required for cell proliferation,^{54,55} again suggesting a context-dependent role for certain members of the PcG.

Together, these observations support the notion that each PcG member either directly or indirectly targets a distinct, specific pathway involved in cell cycle control. At the same time, recent studies suggest that, as with *Bmi1*, the *Mel-18*, *M33/Cbx2*, *Cbx7* (*Pc* homologs) and *Ring1B/Rnf2* proteins^{56,57} may also target the *INK4a/ARF* locus. At least for *Ring1B*, this targeting is probably direct, as the *Ring1B^{-/-}* phenotype is rescued by deleting the *INK4a* locus. *Mel-18^{-/-}* primary mouse fibroblasts also express elevated levels of *INK4a*.³⁸ Nevertheless, it is clear that the *INK4a/ARF* locus is not a general target of PcG proteins, since the anti-proliferative function of *Eed* (*Esc*), which antagonizes *Bmi1* function and negatively regulates the size of the lymphoid and myeloid progenitor cell pools, does not appear to be mediated by the *INK4a/ARF* locus. Moreover, the expression of *INK4a/ARF* is not altered in *Eed* mutants,⁵⁸ suggesting that the effects of *Eed* mutation on cell proliferation are mediated by other pathways. Another example of an *INK4a/ARF*-independent effect on cell proliferation is provided by *Rae28/Mph1*. Mice lacking *Rae28* show profound hematopoietic defects,⁵⁹ with reduced stem cell numbers due to impaired self-renewal and cell proliferation ability.^{60,61} The expression of the *INK4a/ARF* locus, however, is unaffected, even though the patterns of Hox gene expression are clearly disrupted in *Rae28^{-/-}* tissues.⁶¹

Table 1 Role of PcG proteins in cell proliferation

Polycomb member		Targets	Partner	Model	Tissue	Effect on proliferation	Ref
<i>Drosophila</i>	Vertebrates						
Posterior sex combs (Psc)	BMI1 /Bmi1 (PCGF4 /Pcgf4)	p16INK4a (Cdkn2a) /p19ARF		Mouse	Lymphoid cells and primary embryonic fibroblasts (MEFs).	<i>Bmi1</i> deficient cells are impaired with respect to progression into S phase, whereas overexpression of <i>Bmi1</i> allows fibroblast immortalization.	38
			c-Myc	Mouse	Pre-B cells and MEFs	<i>Bmi1</i> prevents c-Myc-induced <i>p19 ARF</i> expression.	96
			E2F6	Human	ML-1 cells	Coimmunoprecipitation experiments show the specific association of E2F6 with BMI1 as well as with MEL-18 and PH1/RAE26 (PHC1).	64
	MEL-18 /Mel-18 (PCGF2 /Pcgf2)	p16INK4a /p19ARF	Cyclin D2	Yeast	Human cDNA library	Identification of MEL-18 as a Cyclin D2 binding protein.	97
				Mouse	Embryonic fibroblasts	<i>p16INK4a</i> and <i>p19ARF</i> mRNA levels are upregulated in MEFs from <i>Mel-18</i> -deficient mouse.	38
				Mouse	B cells	Overexpression of <i>Mel-18</i> results in cell cycle arrest (before entry into S phase) of mature B cells upon B cell receptor stimulation, whereas in <i>Mel-18</i> -deficient mice, the upregulation of c-Myc, <i>cdc25</i> and <i>cdc2/cdk2</i> kinase activities enhances B cell proliferation.	51
Polycomb (Pc)	M33 (Cbx2)	p16INK4a in a E2F dependent manner		Mouse	Embryonic fibroblasts & B and T lymphocyte precursors	S-phase progression is impaired in primary embryonic fibroblasts from M33-deficient mice. These cells have a senescent phenotype associated with an abnormal accumulation of p16INK4a. This defect is bypassed by the expression of a transdominant negative form of E2F. M33 is also critical for the expansion of B and T lymphocyte precursors.	56
	CBX7	p16INK4a (CDKN2A) /p14ARF		Human	Primary fibroblasts	Ectopic expression of CBX7 extends the lifespan of human primary cells by repressing <i>p16INK4a</i> .	57
		p16INK4a /p14ARF	c-Myc	Human	LNCaP and PC-3 prostate cancer cell lines	CBX7 is highly expressed in prostate cancer cell lines and is present at elevated levels in the normal prostate. Specific disruption of CBX7 in normal or cancerous prostate cell lines results in the upregulation of <i>p16INK4a</i> and <i>p14ARF</i> accompanied by the upregulation of <i>p53</i> and <i>p21Cip1</i> and reduced phosphorylation of pRb. CBX7 expression cooperates with c-Myc in rendering LNCaP cells insensitive to growth arrest by androgen receptor inhibition.	98
	PC2 (CBX4 /Cbx4)	CyclinA /cdc2	pRb	Human	CV1,U2OS and C33A cells	Expression of hPC2 in CV-1 and U2OS cells leads to cell proliferation arrest in G2. This arrest is enhanced by the coexpression of pRb. Coimmunoprecipitation experiments in transfected C33A cells show a physical interaction between expressed pRb and hPC2. No interaction is observed between the endogenous pRb and hPC2 proteins.	62
		c-Myc		Mammals	Mammalian cell lines	Interfering with hPC2 function enhances c-Myc expression in several cell lines, whereas overexpressing hPC2 results in c-Myc repression.	53
	dmyc	dmyc	<i>Drosophila</i>	<i>Drosophila</i>	73% of dmyc repression targets require PC for their repression, including the autorepression of dmyc.	99	
	CyclinA		<i>Drosophila</i>	<i>Drosophila</i>	<i>Cyclin A</i> is directly bound by PC and PH in S2 cells and embryonic cycling cells. <i>In vivo</i> , loss of function and overexpression of <i>Pc/ph</i> trigger the up- and downregulation, respectively, of <i>Cyclin A</i> expression.	76	
Sex combs extra (Sce)	Ring1 /RING1	c-jun /c-fos		Mouse	Rat-1 fibroblasts	Rat1a fibroblasts that stably expressed Ring1 specifically overexpressed c-jun and c-fos.	48
			pRb	Human	C33A cells	Coimmunoprecipitation experiments in transfected C33A cells show a physical interaction between expressed pRb and RING1.	62
			E2F6	Human	Hela cells	Purification of an E2F6-containing complex that is active in quiescent cells and contains RING1 and RING2.	63
	RING1B (RNF2)		E2F6	Human	Hela cells	Purification of an E2F6-containing complex that is active in quiescent cells and contains RING1 and RING2.	63
Enhancer of zeste E(z)	EZH2/ EZH1		E2F6	Human	U2OS cells	By affinity purification, a complex including Ezh2 and E2F6 was identified in normal and transformed human cell lines. The association between E2F6 and Ezh2 is specific to proliferating cells.	100
Pipsqueak		pRb together with DNA methylation	Delta	<i>Drosophila</i>	Eye	Psq was identified as an enhancer of the Delta-induced overgrowth phenotype in eyes. Psq is essential for sequence-specific targeting of Polycomb complexes to their targets. Overexpression of Delta and psq induces tumor formation and downregulates Rb expression.	101
Pho	YY1	pRb		Mouse	Myoblast C2C12 cells	YY1 acts as a transcriptional repressor on the Rb promoter in undifferentiated myoblasts.	102

In vertebrates and *Drosophila*, members of the PRC1 and PRC2 have been implicated in the control of cell cycle progression, either through targeting of specific cell cycle genes, or by cooperating with molecules implicated in cell cycle control. The nomenclature used for gene names follows the rules of Flybase for *Drosophila*. For human and mouse genes, we indicate the common usage name, as well as (in brackets) the name following the rules of the HUGO Gene Nomenclature Committee, and the Mouse Genomic Nomenclature Committee.

Consistent with the existence of multiple links between PcG proteins and cell cycle regulators, several PcG members can interact directly with cell cycle factors at the protein level. For example, the human hPC2 protein was suggested to form a complex with E2F and pRb.⁶² Also, several PcG proteins have been shown to form a complex⁶³ containing E2F6 (a transactivation-defective member of the E2F family), the transcriptional regulators Max and Mga (which can bind to c-Myc-responsive genes),^{63,64} NFkappaB,⁶⁵ and Vav (a proto-oncogene that is required for antigen receptor-mediated B- and T-lymphocyte proliferation).^{66,67} In summary, there is extensive evidence that PcG proteins play an important role in orchestrating the intersection between cell proliferation and differentiation (see Table 1 and Fig. 1). Despite all these studies, however, neither the mechanisms by which cell cycle genes are regulated by PcG proteins, nor the global regulatory networks that are established by PcG proteins in order to regulate cell cycle and differentiation, are currently understood.

CYCLIN A: A DIRECT TARGET OF PcG PROTEINS IN DROSOPHILA

Research in vertebrates involves particular difficulties related to functional redundancy and tissue-specificity among genes. Mammals have multiple established or predicted orthologs of PcG

genes, with up to five distinct potential *Pc* homologs and six potential *Psc* homologs.⁶⁸ In recent years, functional conservation among PcG proteins in mammals, plants, and insects has been extensively demonstrated.⁶⁹ We thus decided to use *Drosophila* to search for direct PcG targets among cell cycle genes. In *Drosophila*, removal of a single PcG component is often sufficient to disable the entire repression system. In addition, *Drosophila* is the only organism in which specific DNA sequences have been clearly identified as being targeted by PcG complexes. These sequences are termed PcG response elements (PREs).^{70,71} PREs can be identified because they all share several tractable molecular and genetic properties. For example, PcG proteins directly bind to PREs,^{72,73} and *in vivo* PREs can induce PcG-dependent repression of adjacent white reporter genes⁷⁴ (reviewed in ref. 75). Searching for *Drosophila* PREs in cell cycle genes is therefore one straightforward way to identify direct cell cycle targets of PcG complexes. Using this approach, we found that the *Cyclin A* (*CycA*) gene is bound by PcG members PC and PH. The target sequence is located within the promoter, the first exon and the first intron of the *CycA* gene. Remarkably, this sequence is able to trigger *in vivo* silencing of the white eye color reporter gene in transgenic flies. This repression is PcG-dependent, since it is reversed in PcG mutant backgrounds. In homozygous *Pc*^{-/-} embryos, endogenous *CycA* expression is also upregulated. Conversely, when

Pc and *ph* are overexpressed in dividing embryonic cells, *CycA* expression is downregulated. In both cases, the effect on *CycA* expression is ubiquitous and appears to be cell-autonomous in embryos. Therefore, PcG proteins can act as direct transcriptional repressors of cell cycle genes.⁷⁶ Whether PcG complexes act in collaboration with pRb or with members of E2F family remains to be determined.

Using a combination of immunostaining and FISH (ImmunoFISH) to follow the recruitment of the PC protein to the *CycA* locus during embryonic development, we observed that PC colocalizes with the *CycA* gene in diploid embryonic nuclei. This colocalization increased both in frequency and with respect to the strength of the PC signal as cells progressed through their differentiation programs, reaching a maximum after three zygotic cell divisions. This enrichment paralleled the progressive repression of *CycA* transcription. This result indicates that PcG complexes probably play a role in the differentiation program of the embryo, and is thus in perfect agreement with PcG protein function being required for the establishment of a cellular memory of *CycA* silencing.

At the same time, other results from the same study suggest that the PcG-dependent repression of *CycA* might be dynamic in some contexts. In dividing S2 cultured cells, for example, *Pc* depletion is associated with a lengthening of G₂ phase. This cell cycle effect occurs concomitantly with an increase in *CycA* expression. Because S2 cells continue to divide despite the partial loss of PcG products, this means that *CycA* transcription can be modulated by PcG proteins as the cell cycle progresses, and is not only silenced in conjunction with the exit of cells from mitotic division. How PcG complexes could exert such dynamic regulatory effects is of course a major issue that requires further investigation. Several recent studies, however, have shown that PcG complexes can dynamically respond to signaling cascade pathways in order to establish new cell identities.^{77,78}

Finally, we did not detect binding of PC and PH members at the *CycA* locus on larval polytene chromosomes. This intriguing observation means that the transcriptional repression of *CycA*, which is necessary to drive the endoreplication of the polytene chromosomes, must be either independent of PcG complexes or dependent on PcG proteins other than PC or PH. In the latter case, this would be parallel to what is observed in vertebrates with the *INK4a/ARF* locus, which seems to be targeted by different PcG complexes in different tissues. In summary, the results of our work suggest that the regulation of PcG proteins can be more dynamic and cell-type specific than previously recognized.

DYNAMIC FUNCTION OF PcG PROTEINS AND CELL CYCLE CHECKPOINTS

The activation of checkpoints can arrest cell cycle progression. The connection between *Bmi1* and *INK4a/ARF* is of considerable interest because this locus encodes factors largely controlling the balance between immortalization and senescence in rodent and human primary cells.⁷⁹ Overexpression of *BMI1* in primary human fibroblasts extends the replicative lifespan of the cells by suppressing the p16INK4a-dependent senescence pathway,⁸⁰ and bone marrow progenitors in mice lacking *Bmi1* show an impaired proliferative response to mitogens,⁶ highlighting the potential role of *BMI1* as an integrator of cell cycle checkpoints. Indeed, the *INK4a/ARF* pathway functions as part of a failsafe mechanism that is triggered by inappropriate mitogenic signaling, stress due to unnatural in vitro culture conditions, or the approach of the maximum number of cell

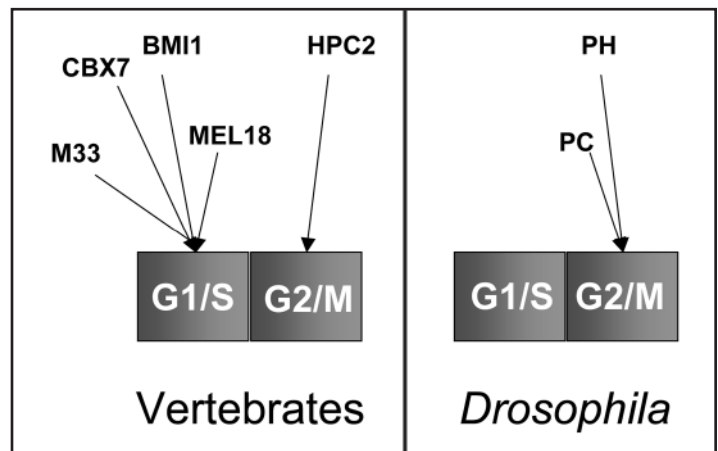


Figure 2. Cell cycle regulatory inputs of PcG members in vertebrates and *Drosophila*. In vertebrates, PcG members have been primarily implicated in the control of the G₁/S transition, with the exception of HPC2. In *Drosophila*, PcG members have been shown to act at the G₂/M transition. Whether *Drosophila* PcG complexes also act at the G₁/S transition remains to be determined. Reciprocally, major control of the G₂/M transition by PcG complexes in vertebrates has not been fully demonstrated and awaits further investigation.

divisions in primary cells. p16INK4a and p19ARF are important players in the pRb (pRb/pINK4a/CycD1) and p53 (pARF/mdm2/p53) pathways,⁸¹ the two main cell cycle control pathways which are frequently targeted in tumorigenesis.

In *Drosophila*, modulating *CycA* levels can be crucial for controlling mitosis, and, indeed, *CycA* is the only essential mitotic cyclin in *Drosophila*.⁸² The function of *CycA* in *Drosophila* is to inhibit a single inhibitor of mitosis, the Fizzy related protein (Fzr), which is present within the Anaphase Promoting Complex.⁸³ In the absence of *CycA*, premature APC activation causes the degradation of substrates that are required for mitotic entry, such as *CycB*. In our experiments, PcG complexes inhibited the accumulation of mitotic *CycA* and, consequently, most likely CDK1 activity. The subtle control of *CycA* levels in vivo by PcG complexes could regulate the kinetics of entry into mitosis. *Drosophila* *CycA* also appears to be necessary for the metaphase delay that occurs after irradiation and to be sufficient for metaphase delays in general.⁸⁴ PcG proteins might therefore represent excellent targets of mitotic checkpoints, as the modulation of PcG activity might permit the regulation of APC activity, a major checkpoint target that is known to be activated by unrepliated or damaged DNA.

The involvement of PcG complexes in checkpoints would indicate that their activity can be modulated. Indeed, several studies in *Drosophila* suggest that PcG-dependent transcriptional repression can be modulated by signalling pathways in vivo or even reverted. Recently, it was shown in living *Drosophila* embryos and wing imaginal discs that PcG complexes are dynamically exchanged, on their target chromatin, within a period of 1–10 minutes, i.e., more than an order of magnitude faster than the cell cycle time.⁸⁵ Although PcG proteins can maintain tissue-specific repression of certain targets through several rounds of cell division, at least some PcG proteins can also dissociate from the chromatin during mitosis and disperse into the cytoplasm in a differential manner. In *Drosophila*, cells from larval leg imaginal discs can be transdetermined from a leg fate to a wing fate in transplantation experiments. This process, named transdetermination, can be easily visualized by the derepression of

the *vestigial* gene, and has been shown to occur in cells in which PcG function is downregulated by the jun kinase pathway.⁷⁸ This result suggests that PRE-silenced genes can be reactivated by new morphogenetic cues and that PcG silencing might thus be modulated in regenerating tissues.

In vertebrates, the *Mel-18*, *Bmi1*, *M33*, and *Rae28* PcG genes have been shown to be rapidly activated upon the antigenic stimulation of lymphoid B cells and are considered to be immediate early genes.^{51,86} Consistent with this, human *BMI1* is activated by c-Myc.³⁴ Moreover, Cited2 (CBP/p300) is induced by growth factors and modulates the expression of *BMI1* and *MEL-18*.⁸⁷

Together, these results indicate that PcG complexes have the necessary features to sense and/or regulate the events triggering cell cycle control. At the same time, the number of cell cycle genes that may be regulated by PcG complexes in vivo is too large to give a good indication of exactly what cell cycle phase may be controlled by the PcG complexes (Fig. 2).

HOW WOULD CELL CYCLE GENES BE SPECIFICALLY TARGETED BY PcG COMPLEXES?

At first glance, it seems paradoxical that some PcG members act as activators of cell cycle progression whereas others act as negative regulators. This could, however, be explained by the ability of PcG complexes to change in composition and, consequently, in their target specificity. The composition and histone substrate targeting of the PRC2 complex have been shown to change during cellular differentiation.⁸⁸ The same might be true of PRC1-type complexes. One such example is provided by an H2A ubiquitin E3 ligase complex that was purified from HeLa cells, called human Polycomb repressive complex1-like (hPRC1L). hPRC1L is composed of three ring-domain containing proteins, RING1, BMI1 and RING1B/RNF2, as well as HPH2/PHC2 and HPC3/CBX8.¹⁸ Interestingly, *RING1B* knock-down inhibits cell growth, implicating this complex in proliferation control.¹⁸ Mice lacking *Bmi1* and *Ring1* show a greatly reduced H2A ubiquitylation level.¹⁷ Although both RING1 and RING1B contribute to H2A ubiquitination, RING1B is the catalytic subunit.⁸⁹ Interestingly, the integrity of PRC1 is not affected by the loss of BMI1 because MEL-18 can substitute for it in the complex. Unlike with BMI1, however, the MEL-18-containing complex is unable to potentiate RING1B E3 ligase activity. This means that, at least in vitro, changing one component of the PRC1 complex is sufficient to radically modify the catalytic activity of RING1B. By extrapolation, the chromatin of PRC1 target genes could also be differentially regulated by BMI1- versus MEL-18-containing complexes.

Another case in point is CBX7. This protein shows parallels with BMI1 in its ability to repress transcription of *INK4a/ARF*.⁵⁷ However, unlike *BMI1*, *CBX7* is a *Pc* homolog rather than a *Psc* homolog. Although CBX7 colocalizes with RING1 in so-called Pc bodies,⁵⁷ it does not colocalize or physically associate with BMI1 nor with HPC2, suggesting that BMI1 and CBX7 function independently of one another. Consistent with this hypothesis, ectopic expression of *BMI1* rescues *CBX7* knockdown-induced premature senescence in mouse embryonic fibroblasts, and ectopic *CBX7* expression rescues premature senescence in *BMI1*^{-/-} fibroblasts.⁵⁷ CBX7 and BMI1 might regulate the same genomic locus (*INK4a/ARF*) as part of separate PRC1 complexes. The analysis of mouse Pc protein homologs corroborates this idea, and suggests that the functional variability of PRC1-type complexes might stem in part

from the different substrate specificities of the various Polycomb class chromodomain proteins in the complexes, particularly with respect to different types of methylated H3 histone lysines or RNA molecules.⁹⁰ Mouse Pc proteins bind differently to methylated histone H3 and RNA, and binding is enriched in facultative heterochromatin. Consistent with the ability of Pc proteins to “read” different histone marks, different PRC2-like complexes are able to “write” different marks.¹¹

Chromatin targeting specificity might also involve specific interactions of PcG proteins with other factors such as DNA binding transcription factors, cell cycle cofactors, or auxiliary factors that can modify core complexes. For instance, E2F6, a weak transcriptional repressor that is preferentially expressed in human bone marrow progenitor cells,⁹¹ can interact with Bmi1 and may be involved in recruiting the complex to target genes such as *p19ARF*.^{64,92} Moreover, hPC2 has been described as a cosuppressor of pRb, acting in concert with E2F to repress the *CycA* and *cdc2* genes in oncogenic cells.⁶²

Another mechanism that could contribute to targeting specificity is the modulation of PcG protein expression levels, which could in turn modify the composition of PcG complexes and thereby alter their affinities for different target sites.⁸⁸ Again, this is based on work on both PRC2 and PRC1 members. Ectopic expression of EZH2 in prostate cells inhibits the transcription of a large cohort of genes, suggesting that EZH2 acts as a general transcriptional repressor in tumor cells. Overexpression of *EZH2* also leads to the accumulation of a novel PRC4 complex that contains the SirT1 histone deacetylase⁸⁸ and differs from the PRC2/3/4 complex. Another likely example of the effects of expression modulation is provided by studies on hPC2. This protein negatively regulates cell proliferation, contrarily to CBX7 and BMI1. The authors propose a model in which PRC1 complexes trigger proliferation or growth arrest depending on the relative amounts of hPC2 and BMI1 in the complexes.⁶⁸

PcG proteins display extensive tissue-specific expression. For example, despite the high degree of homology shared by *Ring1* and *Ring1B*, they show distinct patterns of expression during development. These expression differences are reflected in the differences in the severity of the phenotypes observed when *Ring1* and *Ring1B* are ablated in mice.⁵ *Ring1B* defects are partially rescued by the concomitant ablation of *INK4a/ARF*.⁹³ *Ring1* causes the elevated expression of *c-Fos* and *c-Jun*⁴⁸ (while the effects of Cbx4 are attributable to c-Myc induction). In view of the number of different PcG homologs that are present in human cells, differences between various PcG family members could well determine PRC1 function in specific cell lineages or at different stages of differentiation.^{8,42} Clearly, specific studies will be needed in well-defined systems in order to understand the molecular logic underlying these apparent intricacies.

IS A PcG CELL CYCLE FUNCTION CONSERVED DURING EVOLUTION?

The PcG proteins (particularly PRC2 members) are well conserved during evolution. Although the nature and the degree of conservation of PREs remain largely unknown, because the ability of PcG genes to regulate homeotic genes is highly conserved in evolution it is plausible that their cell cycle regulatory role is also at least partially conserved as well.

Determining the identity of the cell cycle genes that are regulated by PcG complexes is thus a crucial issue. In vertebrates, the strong effects of PcG proteins on proliferation are associated with their ability

to directly or indirectly regulate a broad range of cell cycle genes. In *Drosophila*, the direct targets of PcG members include not only *CycA* but also the *escargot* (*esg*), *elbowB* (*elB*) and *no ocelli* (*noc*) genes, in addition to a p53-like factor encoded by *bifid*.⁹⁴ Interestingly, both *esg* and *elB*, as well as the known PcG target gene *hh*, were coidentified as potential tumor suppressors in a protein overexpression screen.⁹⁵ Finally, recent evidence suggests that *hh* regulates both proliferation and differentiation in the developing *Drosophila* retina.⁹⁶ These examples may be just the tip of the iceberg.

It would also be of great interest to know if invertebrate PcG complexes are able to directly target genes encoding cell cycle inhibitors, as has been proposed for vertebrate PcG complexes. If this were the case, PcG complexes could be postulated to be a part of an evolutionarily conserved "clock" mechanism that could count stem cell divisions, for instance. In the other direction, it would also be interesting to know whether or not vertebrate PcG complexes can directly recognize homologs of the *Drosophila* PcG target genes that are involved in the transcriptional regulation of cell proliferation. For the moment, the only known example of a conserved function for a PcG protein is the regulation of *CycA* by both *Drosophila* and human proteins, an observation that suggests that the function of PcG members in regulating the G₂/M phases might be conserved. In contrast, no available evidence supports a role for *Drosophila* PcG proteins in regulating the G₁/S phases, as is the case in vertebrates (Fig. 2).

An alternative possibility is that the function of PcG proteins in the regulation of both arthropod and chordate cell proliferation may represent an example of convergent evolution. PcG proteins may have shared a set of ancient target genes, and PREs associated with this original set of genes might have then been duplicated. Presuming that Hox and cell cycle control genes were among the ancient target gene pool, the duplication of PREs and their introduction into other genes of the same class might have been positively selected for, as it could allow the evolution of robust gene regulatory systems. This kind of evolution has already been described for the homeotic genes, which are themselves transcriptional regulators and whose conserved homeotic functions do not necessarily target the same genes.

CONCLUSION: CELLULAR MEMORY VERSUS DYNAMIC GENE REGULATION, TOWARDS THE END OF THE POLYCOMB MEMORY DOGMA?

In summary, we have discussed recent evidence suggesting that PcG proteins do much more than maintaining the memory of Hox regulatory patterns. Emerging evidence in *Drosophila* suggests that they can play dynamic regulatory roles. In vertebrates, this ability seems to have been enhanced by the duplication of these genes, with partial divergence of their expression patterns paralleling the emergence of new functions. Indeed, the cellular memory maintenance function might have arisen secondarily in mammals, in which these proteins might be similar to other versatile chromatin cofactors. Concerning the cell cycle and proliferation, there are many hints that PcG proteins regulate these processes across evolution, even if they are not part of the core cell proliferation machinery. Instead, they seem to modulate the function of cell cycle regulators. The challenge now is to dissect the precise molecular mechanisms underlying PcG action. One particularly exciting area of future investigation will be to determine whether the main function of PcG proteins is to regulate the proliferation machine prior to, during, or after key

cell differentiation events, and whether they are also able to fine-tune the expression of cycling cellular components, perhaps by producing a sort of chromatin oscillator. Regardless of the answer, it seems clear that the notion of PcG proteins having a single cellular role in freezing chromatin states for the maintenance of cell fates should be dropped, and that many surprises concerning their dynamic roles in cell division and development are yet to come.

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