Functional analysis of mouse Polycomb group genes

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Abstract. Two groups of genes, the Polycomb group (Pc-G) and trithorax group (trx-G), have been identified in *Drosophila* to provide a transcriptional memory mechanism. They ensure the maintenance of transcription patterns of key regulators such as the *Hox* genes and thereby the correct execution of developmental programmes. Recent data suggest that this memory mechanism is conserved in vertebrates and plants. Here we discuss current insights into the role of mouse Pc-G genes, with a particular focus on the best-studied *Bmi1*, *Mel18* and *M33* genes, as representative examples. Common phenotypes observed in knockout mice mutant for each of these genes indicate an important role for Pc-G genes not only in regulation of *Hox* gene expression and axial skeleton development but also in control of proliferation and survival of haematopoietic cell lineages. Proliferation defects are also observed in other cell lineages derived from these null-mutant mice, and provide new tools to study the impact of Pc-G deregulation on cell cycle control.

Key words. Mouse Polycomb group; *Bmi1*; *Mel18*; *M33*; homeotic transformations; haematopoietic defects; cell cycle.

Mammalian homologues of the Polycomb and trithorax groups

Both in flies and mice the well-conserved homeotic- or homeobox genes play critical roles in implementing developmental decisions and positional information. Notwithstanding the apparent differences in initiation of *Hom/Hox* gene expression patterns, it is evident that in both flies and mice these patterns need to be stably inherited in a cell-autonomous fashion throughout development. In this light, it is not surprising that regulators ensuring such maintenance of *Hox* gene expression are also well conserved. In *Drosophila* two classes of genes have been identified that maintain homeotic gene activity in the appropriate segments throughout development. The Polycomb group (Pc-G) maintains the repressed state in cells where the homeotic gene originally was inactive whereas the trithorax group (trx-G) sustains the active state in cells where the homeotic gene was originally expressed [1–3]. The Antenna paedia complex (ANT-C) and Bithorax complex (BX-C) homeotic genes are properly expressed at early stages in mutant Pc-G embryos, but become misexpressed as the early transcriptional repressors and activators encoded by the gap and pair-rule genes that initiate correct spatial expression patterns of homeotic genes decay. This suggests that Pc-G genes are required for maintenance of the repressed state rather than the establishment of homeotic expression domains.

Drosophila Pc-G gene products form large multiprotein complexes that can bind through *cis*-elements (PREs, or *Polycomb* response elements) to repress specific target genes [4, 5]. Intriguingly, none of the Pc-G proteins analysed this far have been shown to have sequence specific DNA binding capacity. How Pc-G complexes interact with specific target genes remains a major question. The PREs appear to be nonuniform and complex in composition, likely containing multiple independent motifs. This has suggested models in which the clustering of multiple low-affinity binding sites together with multiple protein-protein interactions between Pc-G proteins may add to the inherent stability of Pc-G-mediated gene repression [6]. The counteracting trithorax genes appear to be a more heterogeneous group, and may fall in different categories. The GAGA factor encoded by the *trithorax-like* (*Trl*) gene binds to specific

DNA sequences, and is part of a nucleosome remodelling complex (NURF) capable of displacing nucleosomes from promoter regions [7]. Another example is brahma (brm), part of a well-conserved protein complex. Like their yeast counterpart, named SWI/SNF, brm is involved in disrupting nucleosome structure [8, 9]. Thus, although brm and NURF represent different activities, both facilitate transcription by counteracting repressive effects of nucleosome/chromatin configuration. Interestingly, at least some of the binding sites for trx-G are in close proximity to PREs, suggesting that trx-G and Pc-G are more closely intertwined [36, 83, 91]. A major challenge is to unravel how a critical balance of the widely expressed trx-G and Pc-G complexes is maintained and is capable of keeping specific target genes stably on or off throughout proliferation and development.

A steadily increasing number of mammalian genes have been identified over the last years that share structural similarity to members of the *Drosophila* Pc-G and trx-G (see table 1). To avoid overlap with other contributions to this issue, we will limit our discussion to a representative sample: the *Bmi1*, *Mel18* and *M33* genes. Detailed studies of null mutant or transgenic mice for these Pc-G genes have revealed telling phenotypes. Both gene-specific as well as phenotypes common to all three genes have been observed, and will be discussed below.

Phenotypes of Pc-G-/- mice

Homeotic transformations: common phenotypes

First evidence for a functional conservation of Pc-G action came from the work on the murine *Bmi1* gene, which was originally identified as a collaborator of *c-myc* in lymphomagenesis [10, 11]. Both *Bmi1* and the highly related *Mel18* gene products share regions of homology, encompassing a RING finger and a central domain, with the *Drosophila* Psc and the related Su(z)2 protein [12–14]. *Bmi1* null mutant mice show subtle posterior transformations of the axial skeleton that correlate with subtle anterior shifts of a subset of *Hox* gene expression boundaries at 11.5 to 12.5 days of development [15, 16]. Very similar skeletal transformations have been observed in *Mel18−/−* mice, whereas mice lacking *M33* (a mouse Pc homologue, see table 1) show related but distinct skeletal transformations and malformations in the cervical area [17, 18]. That the expression of only a subset of *Hox* genes is altered in these mutant mice is illustrated by the fact that only the axial skeleton is affected, whereas patterning of the limbs is normal.

The skeletal transformations observed in these single Pc-G gene null mutant mice are clearly less severe and penetrant than the extreme posterior transformations observed in *Drosophila* Pc null mutant flies [19, 20]. This is likely explained by potential redundancy: whereas most *Drosophila* Pc genes appear to be unique, mouse and human Pc genes generally are represented as highly related gene pairs, such as *Mel18/Bmi1*, Enx1/Enx2, *M33/MPc2* and *Hph1/rae28/Hph2* [11, 17, 18, 21–24] (see table 1). A clear demonstration of overlap in function between *Mel18* and *Bmi1* is revealed by the recent generation of *Mel18−/−;Bmi1−/−* double mutants. Such mice die as early embryos around day 9.5 of gestation, with severely affected *Hox* gene expression boundaries (H. Koseki, personal communication). Interestingly, *Mel18+/−;Bmi1−/−* and *Mel18−/−;Bmi1+/−* mice showed intermediate phenotypes, indicating gene dosage effects. Such effects

Table 1. Pc-G genes and trx-G genes in *Drosophila* and mammals.

	Protein mofifs	Mammalian homologues	
Pc-G			Refs:
Polycomb (Pc)	chromodomain	*M33, MPc2,*	69, 70, 21
Posterior sex combs (psc)	RING finger	*bmi1, Mel18*	12–14
Polyhomeotic (ph)	AHH domain, zinc finger	*Mph1/rae28*	87–89
		Hph2,Hph1	23, 24
Enhancer of zeste (E(z))	set domain	*Enx1/EZH2,*	22, 71, 41
		Enx2/EZH1	
Polycomb-like (Pcl)	PHD finger	*M96*	72, 73
Extra sex combs (esc)	WD40 repeat	*eed*	29, 39, 40
Sex combs on midleg (Scm)	AHH domain, zinc fingers		74
trx-G			
Trithorax (trx)	set domain PHD finger	*Mll, ALL*	75–78, 94
Brahma (brm)	bromodomain, DNA-stimulated ATPase domain	*hbrm/mbrm* *BRG1/mBrg1*	79–82
Absent, small or homeotic discs 1 (ash1)	*set domain, PHD finger, AT hooks*		83
Trithorax-like (Trl)	*tramtrack motif, zinc finger*		90
E(var)3-93D	*tramtrack motif*		84
fs(1)h	bromodomain	*RING3*	85, 86

have also been observed previously in *Bmi1* + /− and *Mel18* + /− mice that display weak posterior transformations [15, 17]. Complementary phenotypes were observed in transgenic mice overexpressing *Bmi1* during embryogenesis. These transgenic mice display anterior transformations of the axial skeleton in a gene dose-dependent manner, accompanied by posterior shifts of at least the *Hox*c-5 and *Hox*c-8 expression boundaries [25, 16]. Such dosage effects are also a clear characteristic of *Drosophila* Pc-G genes [26–28].

The dramatically more severe effects observed in the *Mel18*−/−;*Bmi1*−/− double-mutant embryos approaches the extreme null-mutant phenotype of another recently identified Pc-G gene, named *eed*. *Eed* was identified by positional cloning of a classical mouse gastrulation mutant and is highly homologous to *Drosophila esc*. *Eed* null mutant embryos die at mid-gastrulation with disrupted anterior-posterior patterning of the primitive streak, accompanied with ectopic expression of *evx1*, a mouse homologue of the *Drosophila even-skipped* segmentation gene [29]. Again, effects of gene dosage are suggested by the phenotypes of an *eed* hypomorphic mutant that are born with posterior transformations along the axial skeleton, very reminiscent of *Bmi1* null mutant mice. In contrast to the other currently known Pc-G genes in the mouse, the *eed* gene appears to be unique. Taken together, these results may indicate that the *eed* null mutant phenotype reflects a complete loss of Pc-G function, whereas the *Bmi1*, *Mel18* and *M33* single knockouts are partially rescued by their respective homologues. *Eed* null mutants die at mid-gastrulation, which is before most *Hox* genes initially become active. This brings up the question whether mouse Pc-G function is required only to maintain *Hox* gene expression or whether they may also play a role in earlier development, before or at the onset of *Hox* gene expression. This question becomes especially prevalent in considering mechanisms of *Hox* gene regulation by Pc-G genes, since recent results by Duboule and co-workers suggest that a transient slight offset in the timing of initial *Hox* gene activation may permanently alter *Hox* gene expression boundaries [30]. In this regard, it is noteworthy that most Pc-G genes analysed are expressed well before day 9 of gestation. Whereas such an earlier role at first sight may seem at variance with the *Drosophila* data, where Pc-G function appears dispensable during the first 3 h of development, it should be emphasized that many *Drosophila* Pc-G genes have strong maternal contributions that may obscure such early effects [31–33]. Indeed, it has been demonstrated that for correct expression of at least some of the early acting segmentation genes, a subset of the Pc-G and trx-G genes is required [34–38]. A special, early role for *esc* is also suggested in that the function of *esc* is especially required at the transition stage, when early gap- and pair-rule proteins decay and Pc-G complexes need to take over [39, 40]. This could suggest that *esc*, and by analogy *eed*, may be necessary to initiate Pc-G repression.

Homeotic transformations: unique phenotypes

Although the phenotypes observed in *M33*−/−, *Mel18*−/− and *Bmi1*−/− mice are superficially very similar, a detailed analysis indicated subtle differences, revealing unique phenotypes: *Mel18*−/− mice suffer from intestinal blockage due to hypertrophy of smooth muscle [17]. In contrast, *Bmi1*−/− mice develop neurological abnormalities resulting in ataxic gait and hindlimb paralysis. This is likely caused by decreased cell density in the cerebellum and is most pronounced in the molecular layer [15]. *M33*−/− mice display neither of these defects, but have additional skeletal defects, resulting in more deformed cervical vertebrae and characteristic holes in their scapulae [18]. Interestingly, these unique phenotypes are reflected in the particular and unique subsets of *Hox* genes that are affected in each of these mutant mice. Whereas *M33*−/− mice only have a reported 1 p.v. (prevertebrate) anterior shift of *Hox*a-3, *Mel18*−/− mice and *Bmi1*−/− mice show a more extensive overlap in affected *Hox* genes, encompassing 1 p.v. anterior shifts of *Hox*a-5, *Hox*b-6 and *Hox*c-8. However, *Hox*c-6 and *Hox*c-5 are uniquely affected in *Bmi1*−/− mice, while *Hox*a-7 and *Hox*d-4 are only affected in *Mel18*−/− mice [16, 17]. This suggests that Pc-G complexes lacking either Bmi1 or Mel18 may have different affinities or target site specificities for particular *Hox* genes. Alternatively, several Pc-G complexes may exist that vary in constitution for Pc-G proteins such as Mel18 or Bmi1, each having a preferred set of target genes (see also Discussion below). In considering these diverse additional phenotypes, it is important to emphasize that the observed alterations in *Hox* gene expression boundaries are in almost all cases restricted to the paraxial mesoderm. Why no *Hox* gene expression boundary shifts are seen in the neurectoderm is presently unclear, but the neurological abnormalities in *Bmi1*−/− mice together with other pleiotropic phenotypes in the various Pc-G mutant mice suggest that Pc-G genes may regulate other target genes besides *Hox* genes. This is again not unprecedented in *Drosophila*, where pleiotropic phenotypes have also been observed in specific Pc-G gene mutants, involving segmentation, development of the nervous system, chromosome integrity and cell proliferation [41–46, 38, 92]. This suggests we should consider regulation by Pc-G genes to be a more general and widely applied mechanism of 'cellular memory'.

Genetic and biochemical interactions

Genetic interactions can also be observed in mice lacking two nonhomologous Pc-G genes: *M33*−/−;*Bmi1*−/−

double-mutant mice display enhanced posterior transformations of the axial skeleton, as is clearly manifested in 3–4 p.v. anterior shifts of several *Hox* gene expression boundaries, whereas others remain unaffected (K. Kieboom, S. Bel, N. van der Lugt, M. Djabali and M. van Lohuizen, in preparation). Although more severe than the transformations observed in the single knockouts, these effects are less severe than the dramatic phenotypes observed in *Mel18−/−;Bmi1−/−* double mutants or *eed* null mutants likely because both *Bmi1* and *M33* have a close relative (*Mel18* and *MPc2*, respectively). The synergistic interactions between *Bmi1* and *M33* mutants are indicative of their involvement in the same molecular process and could indicate direct interactions of the respective gene products in a larger protein complex. However, the observed synergy at the same time shows that *M33* and *Bmi1* do not act in a precise linear pathway. As pointed out above, this is also reflected in the unique individual phenotypes and affected *Hox* genes in the respective single mutants. How can we explain these genetic interactions and unique individual phenotypes in molecular terms? Part of the answer has come from recent biochemical evidence for larger Pc-G multiprotein complexes in mouse and human cells. Two-hybrid screens and subsequent coimmunoprecipitation experiments revealed specific interactions between Mph1/rae28 and Bmi1 or Mel18, whereas Mph1/rae28 also was found to dimerize through a separate domain [23]. Coimmunoprecipitation experiments also indicated the presence of the Pc homologues M33 and MPc2 in this complex [23, 21], and sucrose density gradient centrifugation experiments suggest that this complex is large (around a million daltons) but rather heterogeneous in size [24]. Is there one or are there multiple Pc-G complexes? The present resolution of centrifugation- and size-exclusion chromatography experiments does not permit a clear conclusion, although the observed size heterogeneity may favour the latter. Clear caveats of such biochemical experiments are that the Pc-G complexes likely interact with chromatin, and biological relevant complexes may not be easily extractable in native form.

How can we reconcile the genetic and phenotypic data on Pc-G mutants, together with the biochemical results, into a speculative working model? The specific binding of Mel18 or Bmi1 to the same domain on Mph1/rae28, the dimerization capacity of Mph1/rae28 and the ability of these complexes to bind to other Pc-G proteins such as M33 and MPc2 together with the unique target specificity of complexes lacking either Mel18 or Bmi1 suggest that the local ratio of Bmi1/Mel18 concentrations may in part determine the affinity for specific targets. This could explain both the observed gene-dosage effects for *Bmi1* and *Mel18*, as well as the ultimate dramatic consequence of 'tipping' the balance in one direction, in the case of *Bmi1* overexpression

leading not only to anterior transformations of the skeleton but also resulting in tumorigenesis (see below). An immediate following question is, How do proteins like Bmi1 and Mel18 determine interaction with specific targets? Unfortunately, at present we know little about the answer. Remarkably, so far all but one of the analysed Pc-G proteins from both mouse and *Drosophila* appear not to bind to DNA in a sequence-specific manner. The exception is Mel18, which was shown to interact in vitro with a specific oligonucleotide, whereas the highly related Bmi1 protein does not bind the same (or other) sequence [47; M. Alkema and M. van Lohuizen, unpublished observations]. It will be of obvious importance to verify the in vivo significance of the Mel18 binding site, as well as to fine-map the protein domain of Mel18 responsible for specific DNA binding. Alternatively, the Pc-G complexes may perhaps recognize other components of chromatin, rather than DNA itself. Given the complex and extensive protein-protein interactions among Pc-G proteins, it is quite possible that specific, high-affinity interactions with targets may depend on intact larger complexes. An interesting precedent for the latter is the recent observation that Bmi1 expressed in transgenic flies is localized to the same polytene binding sites as its *Drosophila* relatives Psc and Su(z)2. This localization depends on the region of high homology between these proteins, that encompasses the N-terminal RING finger and the adjacent conserved domains [48]. Clearly, in order to understand the functional interaction of Pc-G complexes with chromatin, the isolation of relevant *cis*-target sites (i.e. mouse PREs) is eagerly awaited.

Haematopoietic proliferation defects: a link to cell cycle regulation?

Haematopoiesis: common phenotypes

Apart from skeletal transformations, other apparent phenotypes initially observed in *Bmi1−/−* mice are a clear reduction in size and body weight and severe haematopoietic defects. The latter are restricted to the lymphoid and myeloid lineages and are manifested as a reduced cellularity of lymphocytes in bone marrow, spleen and thymus, a progressive reduction of mature B and T cells, and a clear lack of response of lymphocyts and myeloid cells to mitogenic stimulation by a number of interleukins [15]. Very similar phenotypes have since been observed in *Mel18−/−*, *M33−/−* and *Mph1/rae28−/−* mice, clearly indicating that the Pc-G complex plays an important role in regulating haemopoiesis [49, 18, 93]. Interestingly, initially in newborn knockouts all lymphocyte differentiation stages are present, be it at reduced overall cell numbers. This indicates that the effects of loss of *Bmi1*, *Mel18* or *M33* are not so much on differentiation but rather on cell proliferation or survival. The severe progressive lymphopenia and

aberrant differentiation observed in older mice therefore likely represents a secondary effect. Reduced proliferation/survival capacity is also in line with the lack of response, observed in $Bmi1-/-$ and $Mel18-/-$ mice, to specific interleukines: IL-2, IL-4, steel factor (SF), macrophage-colony stimulating factor (M-CSF) and most notably IL-7, whereas the response to IL-3 is not severely affected [15, 49]. In fact, the observed lymphoid defects bear resemblance to the defects observed in either IL-7$-/-$, IL-7-R$\alpha -/-$ or IL-2-R$\gamma -/-$ mice [50–52], suggesting that lack of IL-7 response may be a crucial determinant of the observed effects in Pc-G$-/-$ mice. A clear role of the IL-7 pathway in providing a survival signal for specific lymphocyte subsets, among which the pro-T-cell population, was recently demonstrated [53, 54]. The pro-T-cell compartment is clearly reduced in $Bmi1-/-$ and $Mel18-/-$ mice, whereas in contrast an increase in pro-T and pro-B cells is seen in $Bmi1$ transgenic mice, ultimately leading to a high incidence of B- and T-cell lymphomas [55]. Therefore it will be important to analyse whether loss of Pc-G function leads to decreased lymphocyte survival, perhaps restricted to specific maturation stages. Studies to investigate this are in progress. At what level is the IL-7 pathway affected in Pc-G knockout mice? All known intermediate components of the IL-7 pathway are still expressed at normal levels, and more compellingly, stimulation of knockout lymphocytes with IL-7 still leads to activation of specific signal transducer and activator of transcription (STAT) transcription factors, which are the farthest downstream targets currently known in IL-7 signalling. Thus, Pc-G complexes most probably act downstream of STATs, perhaps on STAT targets [49].

What is causing the proliferation/cell survival defects, and can these defects be related to specific changes in cell cycle regulatory proteins? Several speculative points of view can be considered that are not mutually exclusive. Reduction of Pc-G function may lead to derepression of chromosomal domains, which may trigger a chromosome integrity damage checkpoint, leading to arrest. Alternatively, derepression of specific target genes may cause overexpression of growth-inhibiting genes or could cause an imbalance in mitogenic signalling that could lead to cell cycle arrest. A possible example of the latter is sustained overexpression of the oncogene Ha-ras in primary cells, leading to growth arrest and senescence rather than proliferation [56]. The recent observations that proliferation defects are not restricted to lymphoid cells, but can also be observed in other cell types derived from $M33$ and $Bmi1$ knockout mice, now allows a thorough biochemical investigation of cell cycle regulators, which previously was hampered by the paucity of lymphocytes obtainable from knockouts (M. Djabali, personal communication; M. van Lohuizen and J. Jacobs, unpublished observations).

Preliminary results clearly show that the proliferation defects like those in lymphocytes result from a reduced S-phase entry (J. Jacobs and M. van Lohuizen, unpublished observations). These studies should help to clarify the role of Pc-G proteins in control of cell proliferation.

Haematopoiesis: unique phenotypes

Apart from the common haematopoietic proliferation defects, differences beween the individual Pc-G mutant mice can also be observed. For instance, overexpression of $Bmi1$ in lymphoid cells of Eμ-$Bmi1$ transgenics leads to increased pro-T- and pro-B-cell populations and B- and T-cell lymphomagenesis [25, 55]. Despite the almost 70% identity with $Bmi1$ (see fig. 1), overexpression of $Mel18$ using the same promoter/enhancer combination did not result in any such effects on lymphocyte proliferation (M. Alkema and M. van Lohuizen, unpublished observations). In addition, whereas $Bmi1$ is frequently activated by proviral insertions upon MuLV infection and strongly co-operates with c-Myc in lymphomagenesis, $Mel18$ has never been observed to be targeted by MuLV [55]. In fact, $Mel18$ has been reported to have a 'tumour suppressor' effect, in that overexpression of antisense $Mel18$ RNA leads to a tumorigenic phenotype in NIH/3T3 fibroblasts [47]. However, no tumorigenesis has been reported to occur in either $Mel18+/-$ or $Mel18-/-$ mice, suggesting that the observed effect could be restricted to particular cell lines and does not point to an in vivo tumour-suppressor function of $Mel18$. Nevertheless, these data suggest clearly distinct functions for $Mel18$ and $Bmi1$ in haemopoiesis. This also follows from different responses to mitogenic stimulation of B and T cells derived from either $Bmi1-/-$ or $Mel18-/-$ mice by LPS or concavalin A, respectively: whereas LPS response of splenocytes from newborn $Bmi1-/-$ mice is normal, splenocytes from adult mice have a clear reduced response. A complementary picture arises in $Mel18-/-$ mice, where adult cells appear normal in lipopolysaccharide (LPS) response, but earlier cells are affected [15, 49]. However, from the results discussed above, it is clear that loss of either $Mel18$ or $Bmi1$ similarly affects of signalling by IL-7 and other interleukins. The emerging picture is thus very similar to the skeletal transformation data, in that in haematopoiesis $Mel18$ and $Bmi1$ both also have common or overlapping targets as well as individual targets, which in case of $Bmi1$ overexpression can contribute to tumorigenesis. Obviously, domain-swapping experiments together with structure-function analysis should provide insight into the functional differences between these highly related Pc-G proteins. The latter has recently been performed for $Bmi1$, both mapping domains necessary for skeletal transformation and tumorigenesis in transgenic mice, as well as in vitro, delineating domains necessary for transcriptional re-

pression and subnuclear localization [23, 55, 57, 58]. The results show that the central conserved domain III is essential for repression, skeletal transformations and association with *Mph1/rae28*, whereas the N-terminally located conserved RING finger is necessary for tumorigenesis and subnuclear localization [23, 55, 58]. Intriguingly, transgenic mice overexpressing a mutant *Bmi1* protein harbouring two point mutations in the RING finger still develop anterior transformations of the axial skeleton but do not show an early expansion of pro-T- or pro-B-cell compartments and do not develop lymphomas [23, 55]. These results, together with the observation that the region of homology encompassing the RING finger is necessary to target *Bmi1* to specific Pc-G-binding sites in *Drosophila* [48], suggest that the RING finger is a critical determinant of target site specificity. This holds especially for targets implicated in tumorigenesis by *Bmi1* overexpression which do not overlap with targets implicated in skeletal transformations. The RING finger likely functions as a protein-interaction domain, and we have recently identified two specific *Bmi1*-RING finger-binding proteins (E. Verhoeven and M. van Lohuizen, unpublished observations). Perhaps the function of the RING finger domain is analogous in this respect to the conserved 'chromobox' domain in Polycomb, which has similarly been shown to be necessary for localizing Pc-protein to polytene-binding sites in *Drosophila* [59–61]. Tumorigenesis is not restricted to the Pc-G gene *Bmi1*; also deregulation of the trx-G gene *ALL* is implicated in neoplasia of myeloid cells. This is illustrated by the frequent disruption of ALL by t(4;11) and t(9;11) chromosomal translocations in acute lymphocytic leukemia in infants [75–78, 94].

A question that comes to mind is, Are *Hox* genes also involved in mediating the effects on proliferation of haematopoietic cells? This possibility needs to be investigated, given the clear implication of several *Hox* genes in proliferation and tumorigenesis of haematopoietic cells [62–67]. Likewise, the skeletal defects observed in Pc-G mutant mice could also be caused by alterations in proliferation rates of bone-precursor cells, a process in which *Hox* genes are clearly implicated [68]. Perhaps we should consider Pc-G proteins, much like their trx-G counterparts, as important accessory factors in the proper regulation of expression of target genes of several mitogenic signalling cascades. In this light, it will be important to precisely characterize the effects on cell cycle regulation, which should help in identifying such important target genes.

Conclusions and perspectives

Mouse knockout studies, together with recent biochemical experiments, clearly indicate the functional conservation of Pc-G multiprotein complexes in mammals and demonstrate their role as dose-dependent regulators of axial skeleton identity. A conclusion reached from the results discussed here is that Pc-G complexes in haematopoietic cell lineages function to control a critical balance between proliferation and survival of precursor cells and differentiation into mature cells. Proliferation defects may be more widespread since they also extend to other cell types in Pc-G mutant mice, and may underly the skeletal phenotypes observed. Whether deregulated *Hox* gene expression is at the heart of these effects remains to be investigated. Defining the cell cycle 'action' point and targets of Pc-G complexes in regulating S-phase entry will clearly be of critical importance in understanding Pc-G function in development and in tumorigenesis provoked by deregulation of Pc-G or trx-G genes. Other questions that need to be answered are, How are specific target gene 'expression states' recognized by the Pc-G and trx-G complexes and At what level do the counteracting Pc-G and trx-G complexes compete? From the experiments discussed above it follows that Pc-G target gene specificity can be determined by the relative concentrations of individual components of the complex, such as the Bmi1/Mel18 ratio. This, together with the recent recognition of *Brm/Brg1* as mammalian homologues of *Drosophila brahma*, involved in nucleosome destabilization and remodelling, suggest new entry points to address these questions.

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