BioEssays
www.bioessays-journal.com

How Polycomb-Mediated Cell Memory Deals With a Changing Environment

Variations in PcG complexes and proteins assortment convey plasticity to epigenetic regulation as a response to environment

Federica Marasca, Beatrice Bodega, and Valerio Orlando**

Cells and tissues are continuously exposed to a changing microenvironment, hence the necessity of a flexible modulation of gene expression that in complex organism have been achieved through specialized chromatin mechanisms. Chromatin-based cell memory enables cells to maintain their identity by fixing lineage specific transcriptional programs, ensuring their faithful transmission through cell division; in particular PcG-based memory system evolved to maintain the silenced state of developmental and cell cycle genes. In evolution the complexity of this system have increased, particularly in vertebrates, indicating combinatorial and dynamic properties of Polycomb proteins, in some cases even overflowing outside the cell nucleus. Therefore, their function may not be limited to the imposition of rigid states of genetic programs, but on the ability to recognize signals and allow plastic transcriptional changes in response to different stimuli. Here, we discuss the most novel PcG mediated memory functions in facing and responding to the challenges posed by a fluctuating environment.

1. Introduction

1.1. Epigenetic Memory Is Established in Early Development and Lasts in Adult Tissues: A Long Tale for PcG Proteins

Polycomb group of proteins (PcG), were identified in *Drosophila melanogaster* as developmental regulators of early embryogenesis.[1,2] Structural homology with heterochromatin protein 1 (HP1) and the analogy with clonal inheritance of phenotypic traits typical of heterochromatic position effects, led to the formulation of the founding concept of chromatin-based cell memory and maintenance of cell identity.[3] The combination of genetic and biochemical functional characterization of PcG gene products in various model systems provided evidence for conserved components acting as multiprotein complexes, that maintain the repressive state of genes by various mechanisms, including site specific chemical modification of histones, interaction with RNA Pol II machinery, chromatin fiber compaction, and nuclear architecture (reviewed by Ref. [4]). The repressive function of PcG is counter-balanced by Trithorax group of proteins (TrxG), originally identified as suppressors of PcG mutants, encoding histone modifiers and ATP-dependent chromatin remodelers that maintain the transcriptionally active state of genes.[5] In Drosophila PcG and TrxG proteins bind promoters and other specialized regulatory elements called Cell Memory Module (CMM) that convey epigenetic inheritance through cell division.[6] In mammalian cells PcG proteins bind preferentially CpG islands close to TSS[7] and recent evidence for CMM has been reported in plants.[8]

Two major canonical complexes have been extensively characterized (reviewed in Ref. [9]), PRC2 and PRC1. PRC2 contains the histone methyltransferase activity responsible for histone H3 lysine 27 tri-methylation (H3K27me3). PRC1 binds H3K27me3 and retains H2A lysine 119 ubiquitination (H2AK119ub) activity. The simplified (and not exhaustive) paradigm for Polycomb-mediated transcriptional repression has

Dr. F. Marasca, Dr. B. Bodega
Istituto Nazionale di Genetica Molecolare (INGM) "Romeo and Enrica Invernizzi"
Milan 20122, Italy
E-mail: bodega@ingm.org

Prof. V. Orlando
King Abdullah University of Science and Technology (KAUST)
Environmental Epigenetics Research Program
Biological and Environmental Sciences and Engineering
DivisionThuwal 23955-6900, Saudi Arabia
E-mail: valerio.orlando@kaust.edu.sa

DOI: 10.1002/bies.201700137

included a hierarchical model for PRCs recruitment to target genes, where PRC2 catalyzes H3K27me3 methylation, leading to PRC1 recruitment and chromatin compaction.[10] Besides this established model of PcG mediated gene repression, accumulating evidences indicate a PRC2 independent recruitment of PRC1 (suggesting by non-overlapping Chip-sequencing mapping data)[11–14]; further findings indicate H2AKub dependent PRC2 recruitment[15,16]; however, other reports in developing Drosophila and early mouse embryos, demonstrated a PRC1 mediated repression independent from H2Aub.[17,18]

Genetic evidence of the role of H3K27me3 in cell memory has been firmly demonstrated in Drosophila where mutations in H3K27 residue recapitulated PcG phenotype.[19] Further, recent report showed that H3K27me3 mark can be transmitted transgenerationally and is required also at the beginning of Drosophila embryogenesis.[20]

Mechanistically, besides the role of PRCs in chromatin modification, they contribute also to the formation of multi-looped higher order structures important for maintaining genes silenced state.[21] At a higher order level, as revealed by Hi-C data, H3K27me3 domains match to a subclass of TADs (Topologically Associated Domains), called Polycomb TADs, while PcG proteins appear to cluster giving rise to subnuclear compartments called Polycomb bodies (as reviewed in Refs. [4,9]). The role of PcG in TADs formation remains to be determined as the loss of PRC2 function was shown to have minimal effects on TADs.[22,23]

Additional factors decorate PcG dependent transcriptional regulation and reciprocal recruitment (refer to Refs. [24,25], discussed in the section *Nuclear PRCs Reveal Unpredicted Interactors and Functions*), including DNA binding proteins that contribute to the PcG mediated epigenetic memory.[26] Further, PcG recruitment and function has been also linked to RNA and in particular to long noncoding RNAs (lncRNAs) involved in epigenetic regulation. However, the specificity of RNA binding remains to be clarified, although specific functions in different regulatory contexts can be expected.[27,28]

In evolution, while PcG gene targets have been conserved, PcG proteins diversification appears much greater than previously anticipated, possibly reflecting organisms' complexity.[24,25,29] This led to the rise of a number of variations in complex composition or stoichiometry,[30] molecular function and genome wide target sites, as demonstrated both for PRC1 and PRC2 in neuronal differentiation.[14]

Indeed, extensive ChIP-seq data obtained on a variety of model systems (e.g., ENCODE Consortium[31,32]) demonstrate that high levels of H3K27me3 mark can be bona fide predictive of a repressed state. However, ChIP-seq data on PcG proteins, revealed that the sole presence of individual PcG components at promoters or other *cis*-elements may not be diagnostic of silenced state, but instead of the competence to silence in response to environmental stimuli[33,34] or other functions including transcriptional activation[35] and alternative splicing.[36] This scenario appears to be particularly relevant in post-mitotic cells where chromatin-based cell memory systems do not operate to ensure transmission through cell division but rather to fix ranges of plasticity in tissue specific gene expression programs.

1.2. PRC2s Diversification in Complex Organisms Results From Different, Sub-Stoichiometric Components Engagement

PRC2 consists of four core proteins: Enhancer of zeste (E(z)) with histone methyltransferase (HMT) activity specific for trimethylation of histone H3 lysine 27 (H3K27me3), Extra sex combs (Esc) with an aromatic cage able to bind H3K27me3, Suppressor of zeste 12 (Su(z)12), and the nucleosome remodeling factor Nurf 55 (reviewed in Refs. [4,9]) (**Figure 1**). Drosophila, as well mammalian cells contain stoichiometric levels of these four components, a biochemical parameter critical for PRC2 physiological function. Lower complexity organisms show a partial degree of conservation: *C. neoformans* PRC2 lacks Su(z)12 homologs but instead contains two additional, unrelated proteins, Bnd1 and Cc1; while nematode *C. elegans* shows only E(z) and Esc homologs (MES2 and MES6).[37] During evolution PRC2 components experienced only little gene duplication. Mammals contain two E(z) homologs, Ezh2 or Ezh1 that are developmentally regulated and engaged in alternative PRC2 core complexes[38,39]; Ezh2 is predominant in undifferentiated or proliferating cells, whereas Ezh1 appears to substitute for Ezh2 in post-mitotic cells.[38,39] Additionally, mammalian PRC2 displays four different and

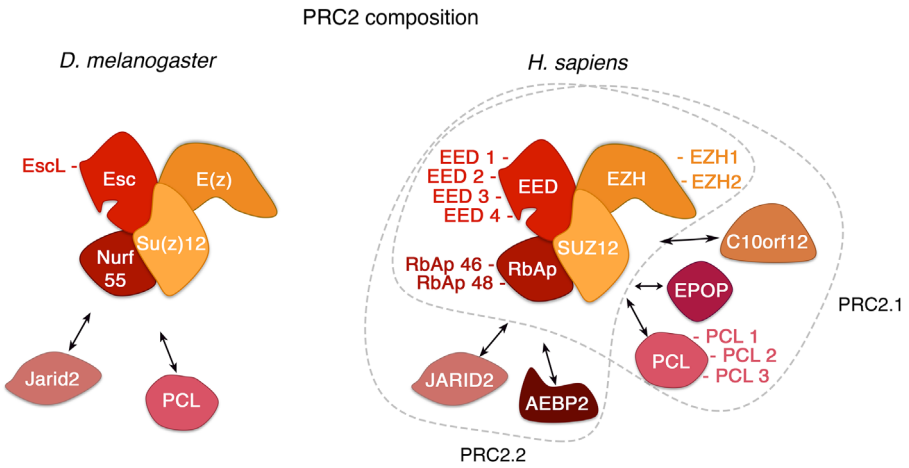

Figure 1. PRC2 configuration in *D. melanogaster* and *H. sapiens*. PRC2 composition and components diversification in *D. melanogaster* (left) and *H. sapiens* (right) are schematically represented. Drosophila PRC2 core components are: Esc or EscL, E(z), Suz(12), and Nurf55; facultative subunits are: Jarid2 and PCL. Human PRC2 core components are EED (1-4), EZH1 or EZH2, Suz12 and RbAp46 or RbAp48; PCL (1-3) or EPOP or C10orf12 facultative subunits give rise to the PRC2.1 complex; Jarid2 or AEBP2 facultative subunits give PRC2.2 complex.

developmentally regulated isoforms of Eed (Esc homolog) produced by specific translational start sites, Suz12 (Su(z)12 homolog) and Rpb46/48 (Nurf55 homologs) (reviewed in Refs. [4,9]) (Figure 1).

In mammals, PRC2 underwent complex diversification because of the engagement of additional facultative subunits that, in sub-stoichiometric ratio and in cell-type dependent fashion contribute to finely modulate its enzymatic activity and/or its recruitment on chromatin.[30,40] In human cells PRC2 exists in two alternative complexes,[40] one defined by the presence of one of the three Polycomb-like homologs (PCLs) (PRC2.1), and the other defined by mutually exclusive AEBP2 and JARID2 (PRC2.2) (Figure 1).[30] PRC2.1 contains two more facultative subunits, C10orf12 and EPOP (Figure 1).[30] Notably, other PRC2-related complexes were reported to contain NAD^+-dependent histone deacetylase Sir2 (in flies) and the histone deacetylase Sirtuin 1 (SIRT1) (in human cancer cells),[41,42] foreseeing a link between cellular metabolic states and transcriptional outputs.

PRC2-Ezh2 evolved toward a much greater complexity in plants, showing significant gene duplications in species as *A. thaliana* with up to 12 homologs of PRC2 components (namely three E(z) homologs, the CLF proteins; two Su(z)12 homologs, the EMF2 proteins; five Nurf55 homologs called MSI1; one Esc homolog renamed FIE),[43] forming at least three distinct complexes involved in different developmental processes.[43] The function of PRC2 in plants has been extensively studied and became a paradigm in environmental epigenetic regulation of vernalization by controlling correct flowering time via silencing of the *FLC* locus in combination with lncRNA (see section 2.5 Ref. [44]).

1.3. PRC1s Diversification in Complex Organisms Mainly Results From Gene Duplication and Expansion

Since the early reports of cloning of PcG homologues in vertebrates,[45] it became clear that PRC1 complexity boosted during evolution by gene duplication and expansion (**Figure 2**). Each of the Drosophila PRC1 protein has at least one extra mammalian homologs and paralogues (Figure 2A,B): RING1 and RING2; three Ph homologues (Polyhomeotic-like protein [PHC1-3]), five Pc homologues (CBX2, CBX4, CBX6, CBX7, and CBX8), two Psc homologues (BMI1 and MEL18, respectively, PCGF4-2) and four other Polycomb group RING finger proteins (PCGFs 1,3,5,6), three Scm homologs (SCMH1/L1/L2) (Figure 2A).[4,9,24]

The PCGF proteins form at least six non-canonical PRC1 (ncPRC1) complexes that do not contain a CBX protein, but other PcG unrelated proteins (Figure 2B).[25,29] In principle, 180 different PRC1 complexes could exist in mammals if all the possible combinations of PRC1 homologous proteins would be able to form, some of them coexisting in a specified cell or at a certain developmental stage.[4,29,46] The molecular function of different versions of PRC1 is unknown and beyond the scope of this review. Indeed, genome wide studies indicate that binding of different sets of PRC1 components can occur in diverse combinations and they can be found at both repressed and active sites.[25] This may explain the complexity of phenotypes

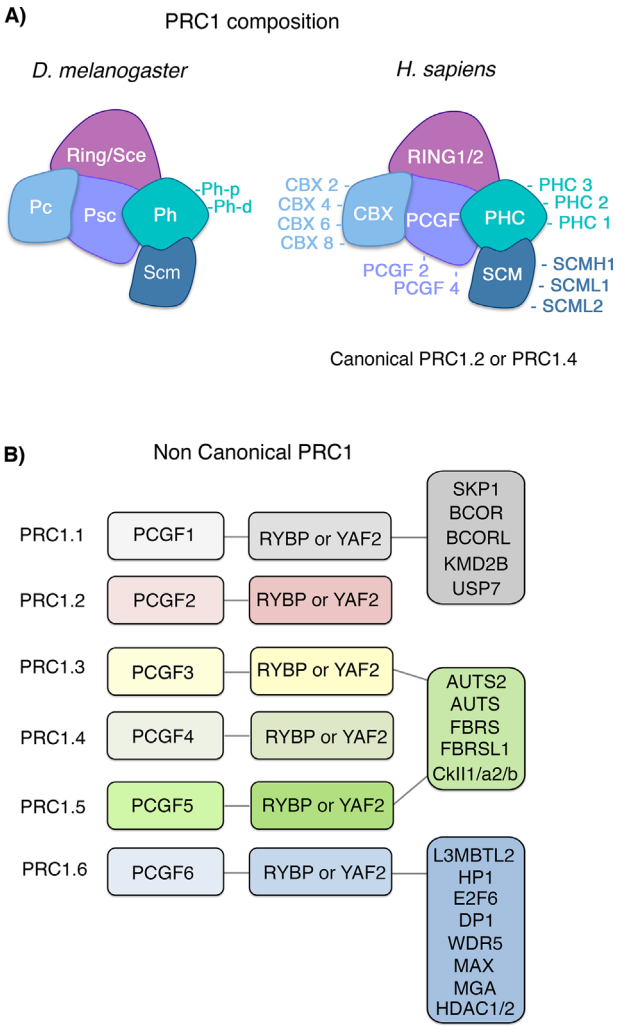

Figure 2. PRC1 configuration in *D. melanogaster* and *H. sapiens*. A) PRC1 composition and components diversification in *D. melanogaster* (left) and *H. sapiens* (right) are schematically represented. Drosophila PRC1 complex is characterized by Pc, Ring or Sce, Ph-p or Ph-d and Psc. Human canonical PRC1 is characterized by CBX (4,6,7,8), RING (1-2), PHCs (1-3), PCGF (2 or 4), and by Scm components (SCMH1, SCML1,2). B) Human non canonical PRC1s (PRC1. 1-6) arise from the interaction of RINGs with one of the PCGFs (1-6) proteins, with RYBP or YAF and other PcG unrelated proteins.

resulting from loss or gain of PRC1 function, including cancer.

In Plants, PRC1 components were only recently identified in *A. thaliana* and they appear highly divergent from animals. PRC1-like RING-finger protein homologues (AtRING1b; AtBMI1) and H2Aub1 function have been reported (reviewed in Ref. [47]). Also proteins belonging to the subfamily of Psc and Pc have been discovered in *A. thaliana*.[47] Interestingly in plants the protein Like-HP1, a homologue of Heterochromatin Protein1 (HP1), binds H3K27me3 instead of H3K9me3 and is a PRC2 interactors.[48]

In evolution PRC1 components appear to have gained diverse modes of action depending on phenotypic requirements.[29]

Indeed, beyond developmental defects and homeotic transformation in flies or embryonic lethality at gastrulation in mice, genetic studies have established a more general role of PcG in cell identity establishment and maintenance.[49–54] During differentiation PRC2 experienced an exchange between Ezh2 and Ezh1,[38] and different supplementary proteins are incorporated to modulate its function and genomic binding.[4] Similarly, PRC1-complex composition (CBX or PCGF proteins) and the corresponding target genes vary in pluripotent ESCs compared to differentiated cells,[55,56] suggesting specific functional properties for different complexes to enable chromatin to be responsive to the environmental changes.

In conclusion, PcG cell memory system appears to build an intricate *scenario* characterized by a profusion of players, including extra nuclear components (see section 3,4 and 5), whose function is not limited to maintain a given states but rather to modulate transcriptional outputs and related phenotypes. Despite decades of intensive studies on PcG, many gaps remain in our understanding of how PRC1 and PRC2 can convey such plasticity to transcriptional programs in particular in adult differentiated cells; in the current review we will summarize some of the latest discovered multifaceted mechanisms by which cells can adapt to environment via Polycomb proteins.

2. How do Polycomb Proteins Sense and Deal With Cellular Micro Environmental State Fluctuations?

Memory is the result of a dynamic process involving learning, codification, and the ability to reproduce the acquired information in the absence of the triggering event. Cells are daily exposed to fluctuations of environmental conditions, the epigenome function is to allow phenotypic variation in terms of transcriptional programs adaptation to the changing environment and to store cell memory throughout life.[57] In the present paragraph we will highlight some of the main aspects related to the PcG memory system and adaptation.

2.1. Polycomb Proteins and Their Connection With Cell Signaling Cascades

Several studies suggest that Serine/Threonine (Ser/Thr) kinases control PcG function by direct PcG proteins phosphorylation or histone modification.[58] Cell-cycle-regulated phosphorylation of EZH2 by Cyclin-dependent kinase 1 (CDK1) and 2 (CDK2) leads to EZH2 degradation and global reduction in H3K27me3 levels. These events impact interaction with lncRNAs and affect regulation of cell cycle progression, differentiation, and tumorigenesis.[59,60] Further, EZH2 can be phosphorylated by Akt kinase (involved in cell growth and nutrient-responsiveness pathways); this modification affects its HMTase activity, leading to decreased H3K27me3 levels and tumor formation.[61] Oncogenic effects are observed also as consequence of Akt-dependent phosphorylation of other PcG like Bmi1, coupled to increased H2A PRC1 ubiquitin-ligase activity,[62] while the Akt-mediate phosphorylation of Mel18 controls Ring1B recruitment to chromatin.[63]

Signal-dependent Ser/Thr kinases were shown to regulate PRC2 directly on chromatin by virtue of a histone modification that disassembles or recruits PRC2 from target genes. MSK2 mediated phosphorylation of H3S28 was shown to promote a switch from H3K27me3 to H3K27ac modifications, antagonizing PRC2-Ezh2 (but not Ezh1) function and affecting cell differentiation.[33] On the contrary, the interaction between mTOR, S6K1, Wnt signaling, and EZH2 seems to strictly coordinate adipogenesis: mTORC-activated S6K1 was shown to act also in the nucleus where it phosphorylates H2BS36. This modification enhances EZH2 recruitment and H3K27me3 on anti-adipogenic genes, blocking *Wnt* expression, a pathway involved in the control of obesity.[64]

Altogether these studies highlight the tight control that cell signaling exert onto PcG in chromatin to control cell fate.

2.2. Polycomb Proteins React to a Variety of Cellular Stresses

Usually signaling pathways are directly initiated by cellular stresses. In mammalian cells, reactive oxygen species (ROS) or serum starvation may modulate PcG proteins localization. Following ROS stress, phosphorylation by MAPK (mitogen activated protein kinase) negatively controls Bmi-1 triggering its detachment from chromatin.[57] Conversely, in skeletal muscle cells, oxidative stress induces increase of intra-nuclear Eed level driving genome-wide activation of PCR2-Ezh1 complex and silencing of specific muscle gene networks.[34] In Cardiac hypertrophy generated upon stress or hormone stimulation, the interaction between the lncRNA Chaer and PRC2, inhibits Ezh2 repressive function at hypertrophic genes leading to their induction.[65]

Furthermore, an important study in Drosophila showed that JNK-dependent negative modulation of intracellular PcG levels accompanies adaptive response to injury to allow tissue regeneration, indicating the importance of epigenome structure modulation in cell survival and tissue repair mechanisms.[66]

PcG dependent adaptation was also demonstrated in response to heat-shock stress and temperature increase. In Drosophila, heat shock induces global gene silencing accompanied by PcG recruitment, histone H3K27me3 modification and the redistribution of architectural proteins facilitating the interaction between enhancer and promoter with PcG proteins.[67] Thus dynamics of PcG system exerts a protective role against stress also by regulating major nuclear architectural rearrangements.[67]

PcG have been shown to bind RNA, but with unclear specificity. Although the role of lncRNA in PRC2 function remains controversial, the interaction with lncRNAs involved in epigenetic regulation has been established (reviewed in Ref. [68]). Beyond lncRNAs, PRC2 Ezh2 and Suz12 subunits have been shown to engage nascent mRNA at promoters,[69] in line with a previously proposed role for PcG in directly interacting with and blocking Pol II processivity.[70,71] Further, an intriguing role for Ezh2 and ncRNA in heat-shock induced stress has been reported in mouse cell lines.[72] This involves a specific interaction between Ezh2 and B2 SINE

retrotransposons RNAs. In detail, B2 RNAs bind RNA Pol II at stress responsive genes maintaining their repression or low levels of expression. Upon heat-induced stress, Ezh2 interacts with B2 SINE RNAs triggering their cleavage and degradation, releasing Pol II and allowing prompt activation of heat responsive genes; this is a novel function for Ezh2 that integrates gene repression and activation, independently from its enzymatic HMTase activity.[72] Another link with stress might be provided by lncRNA MALAT-1 (metastasis-associated lung adenocarcinoma transcript 1). MALAT-1 is a well-characterized lncRNA, stress inducible, involved in speckles formation, a subnuclear interchromatin compartment involved in RNA processing. Recent reports indicated a direct interaction between PRC2 and MALAT-1 and influence on EZH2 oncogenic activity.[73,74] These evidences establish new paradigms linking stress-induced regulation of PcG functions and maintenance of cell identity.

2.3. Polycomb Proteins Safeguard and Guarantee the Epigenetic Inheritance

Epigenetic effects and inheritance refer to the hereditability of phenotypic traits in the absence of the early triggering stimuli. Both mitosis and meiosis are challenging processes where the epigenetic information has to "survive" the complexity of DNA replication, where the epigenomic environment has to be promptly *cis*-restored. Current models of PcG mitotic inheritance envisage cell cycle regulated increase of PcG proteins and histone modifications prior to replication fork passage, to prevent the dilution of the signature,[75] the persistence of PcG components on chromatin during DNA replication and post replicative activity enhancement of PRC2 to restore the H3K27me3 original levels.[4] Moreover, lncRNA may contribute *in cis* to PcG memory function. Interestingly lncRNAs transcribed from opposite strands of Drosophila CMM were shown to mediate alternatively positive or negative effects on PcG silencing.[76] The mechanisms by which these RNAs are involved in epigenetic inheritance remains to be understood.

Transgenerational phenomena imply the recording of individual biological experience in germ cells. In most species, epigenome modifications are reset during gametogenesis, however, some traits can persist and can be stably transmitted throughout generations. While these effects have been observed in plants as well as worms and some fishes, the molecular messengers (e.g., RNA moieties or DNA methylation) that convey the ability of germ cells to record and transmit the experience of a parental organism remain to be identified. Metazoans and in particular mammals, evolved additional mechanisms of epigenome resetting at the beginning of embryogenesis when the entire genome undergoes almost quantitative demethylation and removal of other marks.[77] PcG and TxG proteins are known, from classical studies, to induce transgenerational inheritance of alternative states of transgenes in flies.[6] A role for 3D chromatin contacts has been reported, for which ectopic epialleles carrying different PRC2-dependent H3K27me3 levels were shown to induce stable paramutation effects in the progeny; the stability of these effects was impaired when chromosomal contacts were removed. This inheritance can be passed from both female and male germlines. Importantly, environmental changes can modify the expression of the epialleles, suggesting a putative function for transgenerational inheritance to the phenotypic variation exerted by natural Drosophila populations.[78]

2.4. Polycomb Proteins Functions Are Under the Control of Cellular Metabolic State

In the recent years an important functional relationship between metabolism and epigenetic regulation of gene expression has been established. Indeed, the availability of methyl, acetyl groups and other cofactors is key aspect of how epigenome modifiers sense the cellular microenvironment and how this impacts on physiological and pathological deviations of cell genetic programs.

Metabolism strongly influences acetyl-transferases, in particular HAT (histone acetyl transferase) and Histone deacetylases (HDACs) enzymes, which depend respectively on Acetil-Coa and NAD^+ cofactors. Fluctuations in metabolic states can thus promptly be reflected by changes in chromatin states.[79]

Similarly, the SET-domain containing methyltransferases, as E(z) and EZH1/2, use the biochemical compound S-adenosyl-methionine (SAM) as a methyl group donor to methylate H3K27 residues. The dietary intake of relevant methyl donor precursors, such as methionine, folate and choline, has been strongly linked to changes in cellular methylation potential. Recently, the nuclear accumulation of active SAM synthetase has been demonstrated to correlate with increased PcG-mediated H3K27 trimethylation[80] and mutations of its gene affect position-effect variegation in Drosophila and enhance PcG-induced homeotic phenotypes,[81] suggesting an intimate, but still unexplored, link between PcG proteins regulation and metabolism. This may include also circadian clock regulation in which a pivotal role has been reported for TrxG proteins.[82]

In plants a systematic analysis of histone modifications including H3S10p, H3K27ac, H3K27me3, and H3S28p revealed diurnal changes in short-day optimal and water-deficit conditions, indicating a mechanism in which circadian reduced binding of repressive factors facilitate activating H3K9ac, H3K27ac, and H3S28p chromatin modifications on genes expressed only at a certain time when required or on those that are differentially expressed during the diurnal cycle.[83]

Finally, evidence has been reported about a role for histone modification and in particular H3K9me3/H3K27me3 in diet-induced transgenerational inheritance of obesity.[84] In this context the role of PcG remains to be elucidated.

2.5. Polycomb Proteins Interface With Climate Changing in Plants

One of the first classical connections between PcG proteins and environment is represented by vernalization. To maximize the reproductive success, plants have evolved a sophisticated mechanism called vernalization to regulate the correct timing of developmental flowering switches in respect to the climate. Vernalization is the epigenetic memory of the exposure to past cold winter and is obtained through the PRC2 mediated

regulation of the *FLC* gene (the brake for flowering). In detail, PRC2 complex covers the entire length of *FLC* gene when it is transcribed in young seedlings as they become exposed to the cold; the prolonged cold favors the formation of an alternative PRC2 complex that starts to nucleate and to deposit H3K27me3 on the gene, progressively imposing a rigid repression; exposure to warm changes again the biochemical composition of the complex by recruiting H3K27me3 binding protein LHP1 leading to transcriptional repression stability and maintenance of the *FLC* locus after the winter and favoring the correct flowering process when favorable photo-temperature conditions are present during the subsequent warm season.[85] Vernalization appears to be a cell-autonomous process in which the number of cells that mark the *FLC* locus is a function of the extent of cold time exposure and requires expression of antisense lncRNAs from the locus. In a recent report analysis of cold-induced epigenetic silencing at *FLC* revealed that Polycomb components function in two phases in which reading and writing by PRC2 and LHP1 for H3K27me3 contribute to reinforce the repressive chromatin state in the *FLC* gene body, conveying its long-term stability.[86] Thus the dosage of environmental factors are directly sensed and quantitatively translated by the endogenous chromatin mediated memory systems into an adaptive response.

2.6. Polycomb Proteins Buffer Chromatin Dynamics

Mathematical modeling of Polycomb mediated transcriptional repression try to explain how PcG proteins can buffer and/or properly adapt to external stimuli. Genes targeted by PcG proteins are regulated *in cis* by the presence of histone marks and *in trans* by many diffusible factors as TFs. It seems that PRC2 works in a slow rate of H3K27me3 deposition and that these slow chromatin dynamics is instrumental to filter transient pulses of transcriptional repression and activation (recognized as noise), maintaining epigenetic memory.[87]

The memory can instead be erased in case of assiduous transcriptional activation or repression. These findings highlight a novel function for Polycomb proteins in noise filtering and consequently in buffering the fluctuation of TFs and chromatin dynamics.[87] Other modifiers share such modeling of activity as the Suv39H1/2 responsible for pericentromeric heterochromatin formation, which through a nucleation and looping mechanism establishes and renders heterochromatin robust toward transient environmental perturbation.[88]

3. Nuclear PRCs Reveal Unpredicted Interactors and Functions

The recent literature contains increasing evidence for variegated functions of Polycomb proteins, dependent on the diversity of developmental stages, interacting partners as well as environmental states. Contemporary studies revealed that PcG components (both PRC1 and PRC2) could be associated with actively transcribed gene loci. The liaison between PRC1 complexes and active genes has been recently demonstrated both for pluripotent and differentiating cells where specific subcomplexes appear to exert different functions. In iPSC (Induced Pluripotent Stem Cells) the RYBP PRC1 subunit interacts with the pluripotency transcription factor OCT4, forming an alternative complex required for the activation of the pluripotency program.[89] In mesodermal cells a MEL18/PCGF2-PRC1 interaction is required for cardiac differentiation. This activity occurs in a stage dependent manner by an exchange of subunits that is instrumental to drive sequential repression and activation of genes able to progressively switch the transcriptional program from mESC, to mesodermal commitment and cardiac lineage.[56]

In the central nervous system a PRC1.5–AUTS2 complex has been identified to be essential for active transcription of target genes via recruitment of p300 acetyltransferase[90]; indeed mouse conditional knockout of AUTS2 experiences various developmental defects.

The interaction between PcG and active transcriptional machinery is reported also in tumorigenic contexts. In leukemic cells an shRNA-mediated knockdown screen performed both in mouse model and human primary AML (Acute Myeloid Leukemia) cells revealed an essential role for ncPRC1.1 in sustaining active state of metabolic genes.[91] Similarly a siRNA screening performed in mammary spheres revealed that Cbx8 component could promote tumorigenesis sustaining Notch-network genes expression by regulating H3K4me3 levels.[92]

As mentioned, mammalian cells contain two copies of the Ezh HMT: Ezh2 and Ezh1. While Ezh2 is distinctive of undifferentiated and proliferating cell, Ezh1 although present in mESC cells, appears to be specific for post-mitotic tissues.[38,39] The function of PRC2-Ezh1 complex has been elusive for long time given the viability of Ezh1 knock-out mice, the failure of recovering the lethality effect of Ezh2 knock-out in embryo and a weak HMT activity.[38,50] Recent studies performed in differentiating skeletal muscle cells showed a preferential association of Ezh1 with active promoters, interaction with RNA Pol II and involvement in transcriptional activation of the myogenic program.[33,93] However, formal mechanistic prove that Ezh1 directly controls RNA Pol II is still missing.

Quantitative proteomic analysis in hematopoietic cell lines led to the identification of an Ezh1-Suz12 alternative complex, lacking Eed, associated with actively transcribed genes[35]; the same subcomplex was then demonstrated to work synergistically with the coactivator UXT in response to TNF-α signaling to recruit RNA pol II and regulate NF-κB pathway activation in the nucleus.[94]

Additional interactors related to the transcriptional machinery have been identified. In mESCs the Set2-H3K36me3 enzyme leads to PRC2 mediated H3K27me1 deposition within highly transcribed genes (a modification coupled to RNA polymerase elongation).[95] Moreover, the Elongin BC And Polycomb Repressive Complex 2 Associated Protein (EPOP) regulate transcription at both H3K27me3 repressed and H3K4me3 active domains.[96,97] EPOP knock-out mESCs do not show any altered self renewal and morphology but carry an impaired distribution of H3K4me3, H2Bub and RNA polymerase II, due to EPOP interaction with the H2B deubiquitinase USP7 and impaired control of repressive PRC2-Jarid2 complex.[96,97]

Importantly, PRC2 methyltransferase activity does not seem to be restricted to histone lysines: in mouse cardiac morphogenesis, PRC2-Jarid2 methylates the cardiac transcription factor

GATA4 on lysine 299, preventing its ability to recruit p300 to its targets and impeding their transcription.[98] In T cells development, Ezh2 has been reported to methylate the NKT cell specific transcription factor PLZF, leading to its degradation, regulating immune homeostasis.[99]

HMT independent PRC2 functions can also arise from alternative interactors. Mouse postnatal cardiomyocites display Eed interaction with HDACs, enhancing their catalytic activity; this novel Eed function is an H3K27me3 independent repressive mechanism, crucial for physiological heart function.[100] Similarly in Drosophila, Pc has been demonstrated to interact with the CREB-binding protein (CBP) acetyl transferase, inhibiting its histone acetyltransferase activity.[101]

Furthermore, besides the canonical activities, homologous proteins have developed specific and proper functions as for the PRC1 component CBX2 or for the PRC2-Ezh1 that in contrast to their homologs, are able to perform nucleosome compaction, a fundamental mechanism to maintain gene silencing in mouse development and differentiation.[102,103]

Finally, in this scenario of unpredicted interactors and novel functions, recent evidences for Polycomb recruitment in vertebrates revealed a cooperative, context dependent relationship between DNA methylation, Transcription factors (TFs), RNA binding, histone modifications, and PRCs.[4] In particular, the transcriptional state seems to govern PRC2 binding and H3K27me3 new deposition, this seems to be restricted to unmethylated, nucleosome free, CpG islands, devoid of activating TFs.[104,105] The role of TFs, indeed results elusive as in very specific manner they can even collaborate to recruit PcG proteins on their target sites.[106] Jarid2 instead has been suggested as the subunit able to bridge RNA interaction and PRC2 recruitment on chromatin.[4,107] Also histone modifications can scaffold PRC2 complex on chromatin, as happen for H3K27me3,[108] for H3K9me3,[109] or stabilize its binding as verified for the H3K36me3.[110]

Conclusively, this scenario indicates the complexity of PcG functions depending on interacting partners and cellular contexts.

4. Non-Nuclear Polycomb Functions Represent a Link Between Environmental Sensing and Cellular Adaptation

Besides the established function of Polycomb in the nucleus, increasing evidences indicate non-nuclear functions for PcG proteins, mainly linked to signal transduction and environmental sensing.

A cytoplasmic PRC2-Ezh2 was identified to regulate actin polymerization in response to signaling and surface receptors activation. In detail, fibroblasts depleted of Ezh2 are no longer able to perform actin polymerization in the formation of the dorsal circular ruffles after PDGF mediated stimulation.[111] The same study reported that in T cells the conditional loss of Ezh2, while not affecting their maturation neither the global levels of H3K27me3, severely impacts actin polymerization that follows T cell receptor (TCR) activation, impairing the maturation of the immunological synapse between TCR and APC cell.[111] The peculiar cytosolic function of PRC2 in response to signal transduction in the immune system, appears to be not only restricted to T cells. In the adaptive immunity, in neutrophils and dendritic cells a cytosolic Ezh2 has been described to bridge integrin signaling and adhesion dynamics in the fundamental biological process of immune cells migration. In detail, Ezh2 methylates Talin, one of several proteins that link the cytoplasmic domains of integrin β subunits to actin filaments, and as consequence, its interaction with F actin molecules is disrupted, affecting the turnover of cell adhesion structures.[112] Further, Ezh2 deletion, compromises integrin dependent transendothelian migration of leukocytes, with important implications for immune responses and related pathogenic processes.[112] In analogy, Ezh2 homolog Ezh1, has been described in T cells as a cytosolic molecule, engaged in the signal transduction of TCR through its interaction with the signaling protein Zap70.[113]

These studies suggested unforeseen functions for cytosolic PRC2 components in cell migration, actin and adhesion molecules polymerization, indicating a possible involvement in cancer cells invasiveness, shedding light on potential novel therapeutic approaches.[114]

Besides leucocytes, also prostate epithelial cells contain cytoplasmic Ezh2, which results to be overrepresented in prostate cancer cells affecting re-modeling of actin filaments and fostering tumor invasiveness.[115] Likewise, Ezh2 is overexpressed in gliomas. Interestingly while Ezh2 expression levels are correlating with tumor grade, its intracellular localization correlates with tumor heterogeneity.[116] These data provide important novel insights in metastasis occurrence, chemotherapeutic resistance, and tumor relapse.[116]

The PRC2 core component Eed has been described to shuttle, in a signal-dependent manner, between nuclear and cytosolic compartment. In T cells upon integrin activation or upon HIV-1 Nef mediated signaling, Eed moves from the nucleus to the cytosol, a shuttling that is instrumental for the prompt derepression of specific genes, that have to be transcribed after integrin activation or to promote the transcription of the HIV virus.[117] Eed is required for the TNF (Tumor Necrosis Factor) signaling pathway: after TNF exposure Eed moves from the nucleus to the plasma membrane where functionally couples TNF-Recetor1 to nSMase2.[118] Intriguingly, the extra-nuclear PRC2 localization and functions in metazoan look conserved also in plants: in *A. thaliana* the Eed homolog FIE forms a cytoplasmic complex with the PRC2 histone methyltransferase MEA that is involved in inflorescence development, supporting the concept of an evolutionary diversification of non-nuclear PcG proteins functions.[119]

These studies indicate how "chromatin associated" epigenetic modifiers activity operate also outside the nucleus to control cell structural plasticity in response to environment.

5. Novel PcG Protein Isoforms: New Players in Epigenetic Control and Fine-Tuning of Genes Expression

The complexity of transcriptome output provided by differential promoter usage and alternative splicing, as recently reported by major consortia (e.g., ENCODE, Fantom), is considered a major source of phenotypic diversification in evolution.[120] The

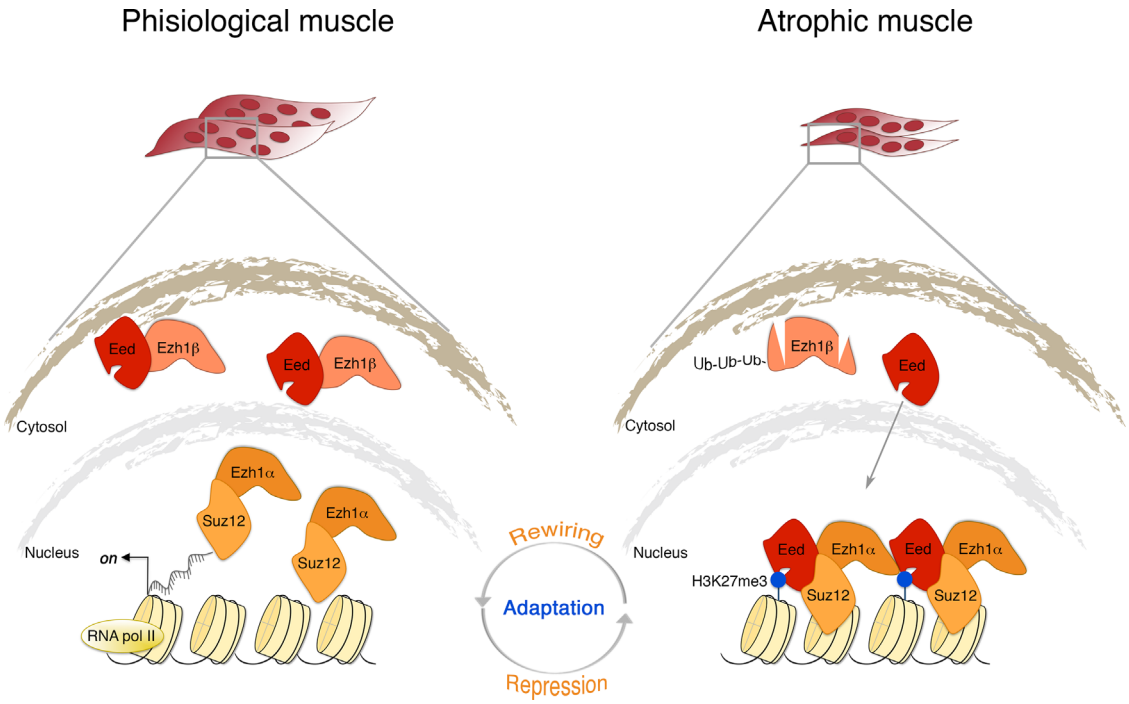

Figure 3. The splicing isoform Ezh1β is a cytosolic stress sensor that controls PRC2–Ezh1α nuclear function. In muscle cells PRC2-Ezh1α formation and activity is kept balanced by the requisitioning of Eed PRC2 core subunit in the cytosol by the alternative isoform Ezh1β (SET domain free) (left). Ezh1β behaves as a stress sensor that upon atrophic stimulation is ubiquitinated and in turn targeted to degradation (right), such event frees Eed to enter in the nucleus and to form a functional PRC2–Ezh1α. This in turn promotes the transcriptional repression of a specific set of muscle genes, inducing an adaptive reversible transcriptional resetting of gene expression.

identification in higher organisms of PcG (and TrxG) members' duplications and splicing variants, broadens the spectrum of mechanisms orchestrating epigenome regulation.

Two Ezh2 splicing variants have been characterized in pancreatic cancer cells, both carrying the catalytic SET domain, showing different gene targets specificity.[121] Ezh1 catalytic SET domain displays 94% of identity with Ezh2. However, its HMT activity in vitro is modest[38] and its role appears to depend on interacting partners and on the physiological state of the cells. Ezh1 has been reported to maintain a repressive state in the absence of Ezh2 in pluripotent, cycling cells[39,122,123] and in male germ cells.[124] In an elegant study performed in developing brain, combined knockout of Ezh1 with Ezh2 revealed cell type and gene network specific defects in neuronal development and maturation resulting in a neurodegenerative phenotype.[125]

Ezh1 remains the predominant, if not the exclusive H3K27-HMTase expressed in differentiated tissues. In a recent study we reported that PRC2-Ezh1 has a key repressive adaptive function specifically exerted in response to oxidative stress.[34] We discovered a novel Ezh1 coding alternative splicing isoform expressed in healthy skeletal muscle fibers, lacking the catalytic SET domain, (Ezh1β). This new protein acts in the cytoplasm (**Figure 3**), where it interacts with Eed, preventing its assembly in a functional, nuclear PRC2-Ezh1α complex. In response to atrophic stimuli Ezh1β is degraded, allowing cytoplasmic Eed to enter the nucleus and form an active PRC2–Ezh1α complex instrumental for adaptive stress response (Figure 3). Importantly, as stress stimuli cease, chromatin state reverts to the previous state.[34]

Splicing variants have been characterized for other PRC2 subunits as for the human *PCL3* that encodes for two isoforms that differ for the absence of one PHD domain (plant homeodomains), they have been characterized in medulloblastoma where they are co-expressed and able to interact with EZH2 but in different sub-complexes, although their function in tumorigenesis remains to be elucidated.[126] Furthermore, as previously anticipated, alternative translational start sites can produce four different Eed isoforms and the incorporation of a distinct Eed into the complex is developmentally regulated and confers HMT substrate specificity in vitro (lysine 26 of histone H1 or lysine 27 of histone H3). In vivo Eed isoform 2, is expressed in cancer cells and undifferentiated embryonic stem cells, and associates with the histone deacetylase Sirt1 in the so-called PRC4 required for the methylation of H1K26.[42,127,128] In the PRC1 world, the *CBX2* subunit undergoes alternative splicing due to different polyadenylation sites, arising two different isoforms, both retaining the chromodomain but the shorter missing the Pc box domain; the latest isoform although unable to join PRC1, self associates and it is reported to repress transcription.[129]

On the contrary, Polycomb proteins have been demonstrated to be also involved in splicing regulation, as for the key factor FRG2 in human mesenchimal stem cells; in detail, lncRNAs recruit Polycomb proteins and the histone demethylase KDM2a to create a specific chromatin setting that promotes an epithelial specific alternative splicing of the *FGR2* gene.[36]

Table 1. Ezh-transcriptional variants in mouse and human.

Enzyme	Uniprot	Protein size (aa)	RNA accession	mRNA length nt	Other transcripts from Ensembl	Length nt	Biotype
Ezh1 *Mm*	P70351-1	747	XM_006532178.3	4198	ENSMUST00000107285.7	4193	Protein coding
	P70351-2	579	AK045374.1	3639	ENSMUST00000146884.7	895	Processed transcript
					ENSMUST00000128974.1	819	Processed transcript
					ENSMUST00000134622.1	726	Processed transcript
					ENSMUST00000138835.7	662	Processed transcript
					ENSMUST00000154211.1	658	Processed transcript
					ENSMUST00000151291.1	373	Processed transcript
Ezh2 *Mm*	Q61188-1	746	NM_007971.2	2665	ENSMUST00000114618.7	2775	Protein coding
	Q61188-2	704	XM_006505524.1	2535	ENSMUST00000092648.12	2115	Protein coding
					ENSMUST00000133043.2	866	Protein coding
					ENSMUST00000204243.2	785	Protein coding
					ENSMUST00000204798.2	1472	Non sense mediated decay
					ENSMUST00000169889.2	666	Non sense mediated decay
					ENSMUST00000167278.7	665	Non sense mediated decay
					ENSMUST00000164006.7	512	Non sense mediated decay
					ENSMUST00000170327.2	834	Retained intron
					ENSMUST00000165492.7	2299	Retained intron
					ENSMUST00000171614.1	1522	Retained intron
					ENSMUST00000203857.1	500	Retained intron
					ENSMUST00000170311.7	483	Retained intron
Ezh1 *Hs*	Q92800-1	747	NM_001991.4	4697	ENST00000415827.6	4608	Protein coding
	Q92800-2	753	NM_001321079.1	4606	ENST00000586382.5	1022	Protein coding
	Q92800-3	707	NM_001321082.1	4577	ENST00000593214.5	745	Protein coding
	Q92800-4	677	AK304835.1	4518	ENST00000586089.5	559	Protein coding
	Q92800-5	608	AK295853.1	4405	ENST00000592492.5	535	Protein coding
					ENST00000588897.5	2283	Non sense mediated decay
					ENST00000586867.5	1031	Non sense mediated decay
					ENST00000588239.5	819	Non sense mediated decay
					ENST00000586935.5	580	Non sense mediated decay
					ENST00000590783.5	595	Processed transcript
					ENST00000590082.1	565	Processed transcript
					ENST00000586103.5	2752	Retained intron
					ENST00000591330.5	2387	Retained intron
					ENST00000585912.5	2295	Retained intron
					ENST00000585550.5	1643	Retained intron
					ENST00000587179.1	804	Retained intron
					ENST00000589846.1	773	Retained intron
					ENST00000586714.1	594	Retained intron
					ENST00000593148.1	591	Retained intron
					ENST00000585455.1	346	Retained intron
Ezh2 *Hs*	Q15910-1	746	NM_001203247.1	2708	ENST00000492143.5	3641	Non sense mediated decay
	Q15910-2	751	NM_004456.4	2723	ENST00000483012.1	1491	Non sense mediated decay
	Q15910-3	707	NM_152998.2	2591	ENST00000498186.5	1876	Retained intron
	Q15910-4	737	NM_001203248.1	2681	ENST00000469631.1	414	Retained intron
	Q15910-5	695	NM_001203249.1	2682			

M. musculus and *H. sapiens* variants of the histone methyltransferases Ezh1 and Ezh2 are listed. We listed protein-coding variants with the respective Uniprot code, expected protein size (number of amino acids, aa), RNA accession code and mRNA size (number of nucleotides, nt). All the other ENSEMBL deposited transcripts are reported, with the respective accession code, RNA size, and predicted biotype.

Finally, the estimated number of *EZH1* and *EZH2* transcripts per TU (transcriptional units) increases between mouse and human (see **Table 1**), possibly matching organism evolution. These findings add further layers of complexity to the combinatorial nature of PcG mediated epigenetic regulation of cellular plasticity.

6. Conclusions and Future Perspectives

Cells, tissues, and organs have constantly to deal with changes of the intra- and external environmental conditions; replication, commitment, differentiation, maintenance of homeostasis, and cell identity memory required chromatin states and gene expression changes. Under such environmental micro-fluctuations epigenetic machineries allow phenotypic adaptation in response to transient signals and, in its absence, retain its memory even without a triggering signal.

Part of the amplitude of cellular functions that multicellular organisms have evolved may reside in the combinatorial nature of protein-protein interactions networks that govern gene expression. PcG epigenetic machinery appears to follow this evolution line, where PRC1 and PRC2 components increased in vertebrates through the expansion in terms of paralogs, homologs, and splicing variants (Table 1).

We describe in this venue classical and novel, non-canonical, epigenetic mechanisms exerted by PRCs in their dealing with a changing environment, trying to figure out the more intrinsic, plastic, nature of the PcG mediated epigenetic memory. This is reached through PcG proteins organization in cell type specific sub-complexes, with definite cellular localization that conducts to the acquisition of unanticipated functions.

The fine dissection of the combinatorial and dynamic nature of PcG cell memory system foresees novel exciting challenges to advance the comprehension of the molecular mechanisms underlying phenotypic variation in response to environmental fluctuations both in physiological and pathological states.

Abbreviations

ATP, Adenosine triphosphate; ChIP-seq, Chromatin Immunoprecipitation Sequencing; CMM, Cell Memory Module; DNA, Deoxyribonucleic Acid; H3K27me3, H3 lysine 27 tri-methylation; H2AK119ub, H2A lysine 119 ubiquitination; HMT activity, Histone Methyltransferase activity; lncRNA, long noncoding RNAs; iPSC, induced Pluripotent Stem Cells; mESC, mouse Embryonic Stem Cells; NAD^+, Nicotinamide Adenine Dinucleotide; RNA, Ribonucleic Acid; ROS, Reactive Oxygen Species; PcG, Polycomb Group of proteins; PRC, Polycomb Repressive Complex; TFs, Transcription Factors; TrxG, Trithorax group of proteins.

Acknowledgements

Beatrice Bodega and Valerio Orlando contributed equally to this work. We thank Chiara Lanzuolo and Alice Cortesi for helpful comments and criticisms on the manuscript. The work is supported by EPIGEN Italian flagship program (to BB), Istituto Nazionale di Genetica Molecolare to BB and King Abdullah University of Science and Technology (KAUST) to VO.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

adaptation, environmental changes, epigenetic memory, evolution, PcG proteins, plasticity, PRC2

Received: July 25, 2017
Revised: January 26, 2018
Published online: March 9, 2018

[1] E. B. Lewis, *Nature* **1978**, *276*, 565.
[2] P. W. Ingham, *J. Embryol. Exp. Morph.* **1985**, *89*, 349.
[3] R. Paro, *Trends. Genet.* **1990**, *6*, 416.
[4] B. Schuettengruber, H. M. Bourbon, L. Di Croce, G. Cavalli, *Cell* **2017**, *171*, 34.
[5] S. J. Geisler, R. Paro, *Development* **2015**, *142*, 2876.
[6] G. Cavalli, R. Paro, *Cell* **1998**, *93*, 505.
[7] D. A. Orlando, M. G. Guenther, G. M. Frampton, R. A. Young, *Genomics* **2012**, *100*, 320.
[8] J. Xiao, R. Jin, X. Yu, M. Shen, J. D. Wagner, A. Pai, C. Song, M. Zhuang, S. Klasfeld, C. He, A. M. Santos, C. Helliwell, J. L. Pruneda-Paz, S. A. Kay, X. Lin, S. Cui, M. F. Garcia, O. Clarenz, J. Goodrich, X. Zhang, R. S. Austin, R. Bonasio, D. Wagner, *Nat. Genet.* **2017**, *49*, 1546.
[9] C. Lanzuolo, V. Orlando, *Annu. Rev. Genet.* **2012**, *46*, 561.
[10] N. J. Francis, R. E. Kingston, C. L. Woodcock, *Science* **2004**, *306*, 1574.
[11] Z. Gao, J. Zhang, R. Bonasio, F. Strino, A. Sawai, F. Parisi, Y. Kluger, D. Reinberg, *Mol. Cell* **2012**, *45*, 344.
[12] L. Tavares, E. Dimitrova, D. Oxley, J. Webster, R. Poot, J. Demmers, K. Bezstarosti, S. Taylor, H. Ura, H. Koide, A. Wutz, M. Vidal, S. Elderkin, N. Brockdorff, *Cell* **2012**, *148*, 664.
[13] M. Yu, T. Mazor, H. Huang, H. T. Huang, K. L. Kathrein, A. J. Woo, C. R. Chouinard, A. Labadorf, T. E. Akie, T. B. Moran, H. Xie, S. Zacharek, I. Taniuchi, R. G. Roeder, C. F. Kim, L. I. Zon, E. Fraenkel, A. B. Cantor, *Mol. Cell* **2012**, *45*, 330.
[14] S. L. Kloet, M. M. Makowski, H. I. Baymaz, L. van Voorthuijsen, I. D. Karemaker, A. Santanach, P. W. Jansen, L. Di Croce, M. Vermeulen, *Nat. Struct. Mol. Biol.* **2016**, *23*, 682.
[15] N. P. Blackledge, A. M. Farcas, T. Kondo, H. W. King, J. F. McGouran, L. L. Hanssen, S. Ito, S. Cooper, K. Kondo, Y. Koseki, T. Ishikura, H. K. Long, T. W. Sheahan, N. Brockdorff, B. M. Kessler, H. Koseki, R. J. Klose, *Cell* **2014**, *157*, 1445.
[16] S. Cooper, M. Dienstbier, R. Hassan, L. Schermelleh, J. Sharif, N. P. Blackledge, V. De Marco, S. Elderkin, H. Koseki, R. Klose, A. Heger, N. Brockdorff, *Cell Rep.* **2014**, *7*, 1456.
[17] A. R. Pengelly, R. Kalb, K. Finkl, J. Muller, *Gene. Dev.* **2015**, *29*, 1487.
[18] R. S. Illingworth, M. Moffat, A. R. Mann, D. Read, C. J. Hunter, M. M. Pradeepa, I. R. Adams, W. A. Bickmore, *Gene. Dev.* **2015**, *29*, 1897.
[19] A. R. Pengelly, O. Copur, H. Jackle, A. Herzig, J. Muller, *Science* **2013**, *339*, 698.
[20] F. Zenk, E. Loeser, R. Schiavo, F. Kilpert, O. Bogdanovic, N. Iovino, *Science* **2017**, *357*, 212.

[21] C. Lanzuolo, V. Roure, J. Dekker, F. Bantignies, V. Orlando, *Nat. Cell Biol*. **2007**, *9*, 1167.
[22] E. P. Nora, B. R. Lajoie, E. G. Schulz, L. Giorgetti, I. Okamoto, N. Servant, T. Piolot, N. L. van Berkum, J. Meisig, J. Sedat, J. Gribnau, E. Barillot, N. Bluthgen, J. Dekker, E. Heard, *Nature* **2012**, *485*, 381.
[23] M. Denholtz, G. Bonora, C. Chronis, E. Splinter, W. de Laat, J. Ernst, M. Pellegrini, K. Plath, *Cell Stem Cell* **2013**, *13*, 602.
[24] Y. B. Schwartz, V. Pirrotta, *Nat. Rev. Genet*. **2013**, *14*, 853.
[25] J. Gil, A. O'Loghlen, *Trends. Cell Biol*. **2014**, *24*, 632.
[26] F. Laprell, K. Finkl, J. Muller, *Science* **2017**, *356*, 85.
[27] C. Davidovich, T. R. Cech, *RNA* **2015**, *21*, 2007.
[28] R. Margueron, D. Reinberg, *Nature* **2011**, *469*, 343.
[29] K. E. Connelly, E. C. Dykhuizen, *Biochim. Biophys. Acta* **2017**, *1860*, 233.
[30] S. Hauri, F. Comoglio, M. Seimiya, M. Gerstung, T. Glatter, K. Hansen, R. Aebersold, R. Paro, M. Gstaiger, C. Beisel, *Cell Rep*. **2016**, *17*, 583.
[31] A. P. Bracken, N. Dietrich, D. Pasini, K. H. Hansen, K. Helin, *Gene. Dev*. **2006**, *20*, 1123.
[32] M. Ku, R. P. Koche, E. Rheinbay, E. M. Mendenhall, M. Endoh, T. S. Mikkelsen, A. Presser, C. Nusbaum, X. Xie, A. S. Chi, M. Adli, S. Kasif, L. M. Ptaszek, C. A. Cowan, E. S. Lander, H. Koseki, B. E. Bernstein, *PLoS Genet*. **2008**, *4*, e1000242.
[33] L. Stojic, Z. Jasencakova, C. Prezioso, A. Stutzer, B. Bodega, D. Pasini, R. Klingberg, C. Mozzetta, R. Margueron, P. L. Puri, D. Schwarzer, K. Helin, W. Fischle, V. Orlando, *Epigenet. Chromatin* **2011**, *4*, 16.
[34] B. Bodega, F. Marasca, V. Ranzani, A. Cherubini, F. Della Valle, M. V. Neguembor, M. Wassef, A. Zippo, C. Lanzuolo, M. Pagani, V. Orlando, *Nat. Struct. Mol. Biol*. **2017**, *24*, 444.
[35] J. Xu, Z. Shao, D. Li, H. Xie, W. Kim, J. Huang, J. E. Taylor, L. Pinello, K. Glass, J. D. Jaffe, G. C. Yuan, S. H. Orkin, *Mol. Cell* **2015**, *57*, 304.
[36] I. Gonzalez, R. Munita, E. Agirre, T. A. Dittmer, K. Gysling, T. Misteli, R. F. Luco, *Nat. Struct. Mol. Biol*. **2015**, *22*, 370.
[37] K. Gall Troselj, R. Novak Kujundzic, D. Ugarkovic, *Clin. Epigenetics* **2016**, *8*, 55.
[38] R. Margueron, G. Li, K. Sarma, A. Blais, J. Zavadil, C. L. Woodcock, B. D. Dynlacht, D. Reinberg, *Mol. Cell* **2008**, *32*, 503.
[39] X. Shen, Y. Liu, Y. J. Hsu, Y. Fujiwara, J. Kim, X. Mao, G. C. Yuan, S. H. Orkin, *Mol. Cell* **2008**, *32*, 491.
[40] D. Holoch, R. Margueron, *Trends Biochem. Sci*. **2017**, *42*, 531.
[41] T. Furuyama, R. Banerjee, T. R. Breen, P. J. Harte, *Curr. Biol*. **2004**, *14*, 1812.
[42] A. Kuzmichev, R. Margueron, A. Vaquero, T. S. Preissner, M. Scher, A. Kirmizis, X. Ouyang, N. Brockdorff, C. Abate-Shen, P. Farnham, D. Reinberg, *Proc. Natl. Acad. Sci. U. S. A*. **2005**, *102*, 1859.
[43] L. Hennig, M. Derkacheva, *Trends. Genet*. **2009**, *25*, 414.
[44] M. Bemer, U. Grossniklaus, *Curr. Opin. Plant. Biol*. **2012**, *15*, 523.
[45] M. van Lohuizen, M. Frasch, E. Wientjens, A. Berns, *Nature* **1991**, *353*, 353.
[46] U. Grossniklaus, R. Paro, *Csh Perspect. Biol*. **2014**, *6*, a019331.
[47] A. Molitor, W. H. Shen, *J. Genet. Genomics* **2013**, *40*, 231.
[48] X. Zhang, S. Germann, B. J. Blus, S. Khorasanizadeh, V. Gaudin, S. E. Jacobsen, *Nat. Struct. Mol. Biol*. **2007**, *14*, 869.
[49] E. M. Morin-Kensicki, C. Faust, C. LaMantia, T. Magnuson, *Genesis* **2001**, *31*, 142.
[50] D. O'Carroll, S. Erhardt, M. Pagani, S. C. Barton, M. A. Surani, T. Jenuwein, *Mol. Cell. Biol*. **2001**, *21*, 4330.
[51] J. W. Voncken, B. A. Roelen, M. Roefs, S. de Vries, E. Verhoeven, S. Marino, J. Deschamps, M. van Lohuizen, *Proc. Natl. Acad. Sci. U. S. A*. **2003**, *100*, 2468.
[52] E. Posfai, R. Kunzmann, V. Brochard, J. Salvaing, E. Cabuy, T. C. Roloff, Z. Liu, M. Tardat, M. van Lohuizen, M. Vidal, N. Beaujean, A. H. Peters, *Gene. Dev*. **2012**, *26*, 920.
[53] L. E. Surface, S. R. Thornton, L. A. Boyer, *Cell Stem Cell* **2010**, *7*, 288.
[54] L. Morey, A. Santanach, L. Di Croce, *Mol. Cell. Biol*. **2015**, *35*, 2716.
[55] K. Klauke, V. Radulovic, M. Broekhuis, E. Weersing, E. Zwart, S. Olthof, M. Ritsema, S. Bruggeman, X. Wu, K. Helin, L. Bystrykh, G. de Haan, *Nat. Cell Biol*. **2013**, *15*, 353.
[56] L. Morey, A. Santanach, E. Blanco, L. Aloia, E. P. Nora, B. G. Bruneau, L. Di Croce, *Cell Stem Cell* **2015**, *17*, 300.
[57] A. Kolybaba, A. K. Classen, *Cell Tissue Res*. **2014**, *356*, 477.
[58] H. E. Niessen, J. A. Demmers, J. W. Voncken, *Epigenet. Chromatin* **2009**, *2*, 10.
[59] S. Kaneko, G. Li, J. Son, C. F. Xu, R. Margueron, T. A. Neubert, D. Reinberg, *Gene. Dev*. **2010**, *24*, 2615.
[60] Y. Wei, Y. H. Chen, L. Y. Li, J. Lang, S. P. Yeh, B. Shi, C. C. Yang, J. Y. Yang, C. Y. Lin, C. C. Lai, M. C. Hung, *Nat. Cell Biol*. **2011**, *13*, 87.
[61] T. L. Cha, B. P. Zhou, W. Xia, Y. Wu, C. C. Yang, C. T. Chen, B. Ping, A. P. Otte, M. C. Hung, *Science* **2005**, *310*, 306.
[62] K. Nacerddine, J. B. Beaudry, V. Ginjala, B. Westerman, F. Mattiroli, J. Y. Song, H. van der Poel, O. B. Ponz, C. Pritchard, P. Cornelissen-Steijger, J. Zevenhoven, E. Tanger, T. K. Sixma, S. Ganesan, M. van Lohuizen, *J. Clin. Invest*. **2012**, *122*, 1920.
[63] S. Elderkin, G. N. Maertens, M. Endoh, D. L. Mallery, N. Morrice, H. Koseki, G. Peters, N. Brockdorff, K. Hiom, *Mol. Cell* **2007**, *28*, 107.
[64] S. A. Yi, S. H. Um, J. Lee, J. H. Yoo, S. Y. Bang, E. K. Park, M. G. Lee, K. H. Nam, Y. J. Jeon, J. W. Park, J. S. You, S. J. Lee, G. U. Bae, J. W. Rhie, S. C. Kozma, G. Thomas, J. W. Han, *Mol. Cell* **2016**, *62*, 443.
[65] Z. Wang, X. J. Zhang, Y. X. Ji, P. Zhang, K. Q. Deng, J. Gong, S. Ren, X. Wang, I. Chen, H. Wang, C. Gao, T. Yokota, Y. S. Ang, S. Li, A. Cass, T. M. Vondriska, G. Li, A. Deb, D. Srivastava, H. T. Yang, X. Xiao, H. Li, Y. Wang, *Nat. Med*. **2016**, *22*, 1131.
[66] N. Lee, C. Maurange, L. Ringrose, R. Paro, *Nature* **2005**, *438*, 234.
[67] L. Li, X. Lyu, C. Hou, N. Takenaka, H. Q. Nguyen, C. T. Ong, C. Cubenas-Potts, M. Hu, E. P. Lei, G. Bosco, Z. S. Qin, V. G. Corces, *Mol. Cell* **2015**, *58*, 216.
[68] M. Kretz, G. Meister, *Mol. Cell* **2014**, *55*, 157.
[69] S. Kaneko, J. Son, R. Bonasio, S. S. Shen, D. Reinberg, *Gene. Dev*. **2014**, *28*, 1983.
[70] G. I. Dellino, Y. B. Schwartz, G. Farkas, D. McCabe, S. C. Elgin, V. Pirrotta, *Mol. Cell* **2004**, *13*, 887.
[71] A. Breiling, B. M. Turner, M. E. Bianchi, V. Orlando, *Nature* **2001**, *412*, 651.
[72] A. Zovoilis, C. Cifuentes-Rojas, H. P. Chu, A. J. Hernandez, J. T. Lee, *Cell* **2016**, *167*, 1788.
[73] S. H. Kim, S. H. Kim, W. I. Yang, S. J. Kim, S. O. Yoon, *Oncotarget* **2017**, *8*, 31305.
[74] D. Wang, L. Ding, L. Wang, Y. Zhao, Z. Sun, R. J. Karnes, J. Zhang, H. Huang, *Oncotarget* **2015**, *6*, 41045.
[75] C. Lanzuolo, F. Lo Sardo, A. Diamantini, V. Orlando, *PLoS Genet* **2011**, *7*, e1002370.
[76] V. A. Herzog, A. Lempradl, J. Trupke, H. Okulski, C. Altmutter, F. Ruge, B. Boidol, S. Kubicek, G. Schmauss, K. Aumayr, M. Ruf, A. Pospisilik, A. Dimond, H. B. Senergin, M. L. Vargas, J. A. Simon, L. Ringrose, *Nat. Genet*. **2014**, *46*, 973.
[77] E. Heard, R. A. Martienssen, *Cell* **2014**, *157*, 95.
[78] F. Ciabrelli, F. Comoglio, S. Fellous, B. Bonev, M. Ninova, Q. Szabo, A. Xuereb, C. Klopp, A. Aravin, R. Paro, F. Bantignies, G. Cavalli, *Nat. Genet*. **2017**, *49*, 876.
[79] M. A. Reid, Z. Dai, J. W. Locasale, *Nat. Cell Biol*. **2017**, *19*, 1298.
[80] E. Reytor, J. Perez-Miguelsanz, L. Alvarez, D. Perez-Sala, M. A. Pajares, *Faseb J*. **2009**, *23*, 3347.
[81] J. Larsson, J. Zhang, A. Rasmuson-Lestander, *Genetics* **1996**, *143*, 887.

[82] L. Aguilar-Arnal, S. Katada, R. Orozco-Solis, P. Sassone-Corsi, *Nat. Struct. Mol. Biol*. **2015**, *22*, 312.
[83] K. Baerenfaller, H. Shu, M. Hirsch-Hoffmann, J. Futterer, L. Opitz, H. Rehrauer, L. Hennig, W. Gruissem, *Plant Cell. Environ*. **2016**, *39*, 2557.
[84] A. Ost, A. Lempradl, E. Casas, M. Weigert, T. Tiko, M. Deniz, L. Pantano, U. Boenisch, P. M. Itskov, M. Stoeckius, M. Ruf, N. Rajewsky, G. Reuter, N. Iovino, C. Ribeiro, M. Alenius, S. Heyne, T. Vavouri, J. A. Pospisilik, *Cell* **2014**, *159*, 1352.
[85] D. C. Baulcombe, C. Dean, *Csh Perspect. Biol*. **2014**, *6*, a019471.
[86] H. Yang, S. Berry, T. S. G. Olsson, M. Hartley, M. Howard, C. Dean, *Science* **2017**, *357*, 1142.
[87] S. Berry, C. Dean, M. Howard, *Cell Syst*. **2017**, *4*, 445.
[88] K. Muller-Ott, F. Erdel, A. Matveeva, J. P. Mallm, A. Rademacher, M. Hahn, C. Bauer, Q. Zhang, S. Kaltofen, G. Schotta, T. Hofer, K. Rippe, *Mol. Syst. Biol*. **2014**, *10*, 746.
[89] H. Li, P. Lai, J. Jia, Y. Song, Q. Xia, K. Huang, N. He, W. Ping, J. Chen, Z. Yang, J. Li, M. Yao, X. Dong, J. Zhao, C. Hou, M. A. Esteban, S. Gao, D. Pei, A. P. Hutchins, H. Yao, *Cell Stem Cell* **2017**, *20*, 571.
[90] Z. Gao, P. Lee, J. M. Stafford, M. von Schimmelmann, A. Schaefer, D. Reinberg, *Nature* **2014**, *516*, 349.
[91] V. van den Boom, H. Maat, M. Geugien, A. Rodriguez Lopez, A. M. Sotoca, J. Jaques, A. Z. Brouwers-Vos, F. Fusetti, R. W. Groen, H. Yuan, A. C. Martens, H. G. Stunnenberg, E. Vellenga, J. H. Martens, J. J. Schuringa, *Cell Rep*. **2016**, *14*, 332.
[92] C. Y. Chung, Z. Sun, G. Mullokandov, A. Bosch, Z. A. Qadeer, E. Cihan, Z. Rapp, R. Parsons, J. A. Aguirre-Ghiso, E. F. Farias, B. D. Brown, A. Gaspar-Maia, E. Bernstein, *Cell Rep*. **2016**, *16*, 472.
[93] K. Mousavi, H. Zare, A. H. Wang, V. Sartorelli, *Mol. Cell* **2012**, *45*, 255.
[94] S. K. Su, C. Y. Li, P. J. Lei, X. Wang, Q. Y. Zhao, Y. Cai, Z. Wang, L. Li, M. Wu, *J. Cell. Sci*. **2016**, *129*, 2343.
[95] K. J. Ferrari, A. Scelfo, S. Jammula, A. Cuomo, I. Barozzi, A. Stutzer, W. Fischle, T. Bonaldi, D. Pasini, *Mol. Cell* **2014**, *53*, 49.
[96] R. Liefke, V. Karwacki-Neisius, Y. Shi, *Mol. Cell* **2016**, *64*, 659.
[97] M. Beringer, P. Pisano, V. Di Carlo, E. Blanco, P. Chammas, P. Vizan, A. Gutierrez, S. Aranda, B. Payer, M. Wierer, L. Di Croce, *Mol. Cell* **2016**, *64*, 645.
[98] A. He, X. Shen, Q. Ma, J. Cao, A. von Gise, P. Zhou, G. Wang, V. E. Marquez, S. H. Orkin, W. T. Pu, *Gene. Dev*. **2012**, *26*, 37.
[99] A. Vasanthakumar, D. Xu, A. T. Lun, A. J. Kueh, K. P. van Gisbergen, N. Iannarella, X. Li, L. Yu, D. Wang, B. R. Williams, S. C. Lee, I. J. Majewski, D. I. Godfrey, G. K. Smyth, W. S. Alexander, M. J. Herold, A. Kallies, S. L. Nutt, R. S. Allan, *EMBO Rep*. **2017**, *18*, 619.
[100] S. Ai, Y. Peng, C. Li, F. Gu, X. Yu, Y. Yue, Q. Ma, J. Chen, Z. Lin, P. Zhou, H. Xie, T. W. Prendiville, W. Zheng, Y. Liu, S. H. Orkin, D. Z. Wang, J. Yu, W. T. Pu, A. He, *eLife* **2017**, *6*, 1005.
[101] F. Tie, R. Banerjee, C. Fu, C. A. Stratton, M. Fang, P. J. Harte, *Proc. Natl. Acad. Sci. U. S. A*. **2016**, *113*, E744.
[102] M. S. Lau, M. G. Schwartz, S. Kundu, A. J. Savol, P. I. Wang, S. K. Marr, D. J. Grau, P. Schorderet, R. I. Sadreyev, C. J. Tabin, R. E. Kingston, *Science* **2017**, *355*, 1081.
[103] J. Son, S. S. Shen, R. Margueron, D. Reinberg, *Gene. Dev*. **2013**, *27*, 2663.
[104] S. J. van Heeringen, R. C. Akkers, I. van Kruijsbergen, M. A. Arif, L. L. Hanssen, N. Sharifi, G. J. Veenstra, *Genome Res*. **2014**, *24*, 401.
[105] E. M. Riising, I. Comet, B. Leblanc, X. Wu, J. V. Johansen, K. Helin, *Mol. Cell* **2014**, *55*, 347.
[106] L. Ringrose, *Curr. Opin. Cell Biol*. **2007**, *19*, 290.
[107] S. Kaneko, R. Bonasio, R. Saldana-Meyer, T. Yoshida, J. Son, K. Nishino, A. Umezawa, D. Reinberg, *Mol. Cell* **2014**, *53*, 290.
[108] T. I. Lee, R. G. Jenner, L. A. Boyer, M. G. Guenther, S. S. Levine, R. M. Kumar, B. Chevalier, S. E. Johnstone, M. F. Cole, K. Isono, H. Koseki, T. Fuchikami, K. Abe, H. L. Murray, J. P. Zucker, B. Yuan, G. W. Bell, E. Herbolsheimer, N. M. Hannett, K. Sun, D. T. Odom, A. P. Otte, T. L. Volkert, D. P. Bartel, D. A. Melton, D. K. Gifford, R. Jaenisch, R. A. Young, *Cell* **2006**, *125*, 301.
[109] C. Mozzetta, J. Pontis, L. Fritsch, P. Robin, M. Portoso, C. Proux, R. Margueron, S. Ait-Si-Ali, *Mol. Cell* **2014**, *53*, 277.
[110] C. Ballare, M. Lange, A. Lapinaite, G. M. Martin, L. Morey, G. Pascual, R. Liefke, B. Simon, Y. Shi, O. Gozani, T. Carlomagno, S. A. Benitah, L. Di Croce, *Nat. Struct. Mol. Biol*. **2012**, *19*, 1257.
[111] I. H. Su, M. W. Dobenecker, E. Dickinson, M. Oser, A. Basavaraj, R. Marqueron, A. Viale, D. Reinberg, C. Wulfing, A. Tarakhovsky, *Cell* **2005**, *121*, 425.
[112] M. Gunawan, N. Venkatesan, J. T. Loh, J. F. Wong, H. Berger, W. H. Neo, L. Y. Li, M. K. La Win, Y. H. Yau, T. Guo, P. C. See, S. Yamazaki, K. C. Chin, A. R. Gingras, S. G. Shochat, L. G. Ng, S. K. Sze, F. Ginhoux, I. H. Su, *Nat. Immunol*. **2015**, *16*, 505.
[113] M. Ogawa, Y. Hiraoka, S. Aiso, *Immunol. Lett*. **2003**, *86*, 57.
[114] R. Prinjha, A. Tarakhovsky, *Gene. Dev*. **2013**, *27*, 1731.
[115] R. J. Bryant, N. A. Cross, C. L. Eaton, F. C. Hamdy, V. T. Cunliffe, *Prostate* **2007**, *67*, 547.
[116] A. Natsume, M. Ito, K. Katsushima, F. Ohka, A. Hatanaka, K. Shinjo, S. Sato, S. Takahashi, Y. Ishikawa, I. Takeuchi, H. Shimogawa, M. Uesugi, H. Okano, S. U. Kim, T. Wakabayashi, J. P. Issa, Y. Sekido, Y. Kondo, *Cancer Res*. **2013**, *73*, 4559.
[117] V. Witte, B. Laffert, O. Rosorius, P. Lischka, K. Blume, G. Galler, A. Stilper, D. Willbold, P. D'Aloja, M. Sixt, J. Kolanus, M. Ott, W. Kolanus, G. Schuler, A. S. Baur, *Mol. Cell* **2004**, *13*, 179.
[118] S. Philipp, M. Puchert, S. Adam-Klages, V. Tchikov, S. Winoto-Morbach, S. Mathieu, A. Deerberg, L. Kolker, N. Marchesini, D. Kabelitz, Y. A. Hannun, S. Schutze, D. Adam, *Proc. Natl. Acad. Sci. U. S. A*. **2010**, *107*, 1112.
[119] M. Oliva, Y. Butenko, T. F. Hsieh, O. Hakim, A. Katz, N. I. Smorodinsky, D. Michaeli, R. L. Fischer, N. Ohad, *J. Exp. Bot*. **2016**, *67*, 6111.
[120] S. Djebali, C. A. Davis, A. Merkel, A. Dobin, T. Lassmann, A. Mortazavi, A. Tanzer, J. Lagarde, W. Lin, F. Schlesinger, C. Xue, G. K. Marinov, J. Khatun, B. A. Williams, C. Zaleski, J. Rozowsky, M. Roder, F. Kokocinski, R. F. Abdelhamid, T. Alioto, I. Antoshechkin, M. T. Baer, N. S. Bar, P. Batut, K. Bell, I. Bell, S. Chakrabortty, X. Chen, J. Chrast, J. Curado, T. Derrien, J. Drenkow, E. Dumais, J. Dumais, R. Duttagupta, E. Falconnet, M. Fastuca, K. Fejes-Toth, P. Ferreira, S. Foissac, M. J. Fullwood, H. Gao, D. Gonzalez, A. Gordon, H. Gunawardena, C. Howald, S. Jha, R. Johnson, P. Kapranov, B. King, C. Kingswood, O. J. Luo, E. Park, K. Persaud, J. B. Preall, P. Ribeca, B. Risk, D. Robyr, M. Sammeth, L. Schaffer, L. H. See, A. Shahab, J. Skancke, A. M. Suzuki, H. Takahashi, H. Tilgner, D. Trout, N. Walters, H. Wang, J. Wrobel, Y. Yu, X. Ruan, Y. Hayashizaki, J. Harrow, M. Gerstein, T. Hubbard, A. Reymond, S. E. Antonarakis, G. Hannon, M. C. Giddings, Y. Ruan, B. Wold, P. Carninci, R. Guigo, T. R. Gingeras, *Nature* **2012**, *489*, 101.
[121] A. Grzenda, G. Lomberk, P. Svingen, A. Mathison, E. Calvo, J. Iovanna, Y. Xiong, W. Faubion, R. Urrutia, *Epigenet. Chromatin* **2013**, *6*, 3.
[122] I. Hidalgo, A. Herrera-Merchan, J. M. Ligos, L. Carramolino, J. Nunez, F. Martinez, O. Dominguez, M. Torres, S. Gonzalez, *Cell Stem Cell* **2012**, *11*, 649.
[123] E. Ezhkova, W. H. Lien, N. Stokes, H. A. Pasolli, J. M. Silva, E. Fuchs, *Gene. Dev*. **2011**, *25*, 485.
[124] W. Mu, J. Starmer, Y. Shibata, D. Yee, T. Magnuson, *Dev. Biol*. **2017**, *424*, 198.
[125] M. von Schimmelmann, P. A. Feinberg, J. M. Sullivan, S. M. Ku, A. Badimon, M. K. Duff, Z. Wang, A. Lachmann, S. Dewell, A. Ma'ayan, M. H. Han, A. Tarakhovsky, A. Schaefer, *Nat. Neurosci*. **2016**, *19*, 1321.

[126] G. Boulay, C. Rosnoblet, C. Guerardel, P. O. Angrand, D. Leprince, *Biochem. J*. **2011**, *434*, 333.
[127] S. Y. Kim, J. M. Levenson, S. Korsmeyer, J. D. Sweatt, A. Schumacher, *J. Biol. Chem*. **2007**, *282*, 9962.
[128] A. Kuzmichev, T. Jenuwein, P. Tempst, D. Reinberg, *Mol. Cell* **2004**, *14*, 183.
[129] P. Volkel, P. Le Faou, J. Vandamme, D. Pira, P. O. Angrand, *Epigenetics* **2012**, *7*, 482.