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Chiara Lanzuolo
Beatrice Bodega *Editors*

Polycomb Group Proteins

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Polycomb Group Proteins

Methods and Protocols

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Preface

Investigating the Polycomb Group of Proteins: Technologies à la Carte

In eukaryotic cells, genomic DNA is packaged in a highly regulated conformation inside the nucleus. This ordered shape consists of multiple levels of epigenetic regulation resulting from dynamic interactions between the genome, chromatin modifiers, and various species of noncoding RNAs. The overall set of DNA, histone modification, and chromatin regulators define an epigenome, which is cell specific and regulates the transcriptome, determining the cell identity (reviewed in [1]).

The Polycomb group of proteins (PcG proteins) is one of the most studied families of transcriptional repressors which act on chromatin at various levels of regulation, from modification of histone tails to modulation of DNA-DNA association (reviewed in [2]). To date, several PcG complexes have been purified and characterized in various organisms, revealing that the combinatorial association of PcG proteins and their co-regulators determines their enzymatic functions and target's specificity. In mammals, the best-characterized complexes are Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2) that can act synergistically or independently of each other and are responsible for the H2AK119ub and H3K27me3 histone signature placements, respectively (reviewed in [3]). In addition to histone modifications, PcG proteins modulate the folding of chromatin in specific higher order structures, which favor the maintenance of genes repression (reviewed in [2, 4]). Hence, in the nuclear space, PcG proteins are organized into aggregates called PcG bodies, mediated by intrinsic and extrinsic protein-protein interactions [1] whose assembly mirrors the chromatin architecture of PcG clustered targets. Interestingly, recent findings have shown that PcG proteins are also able to crosstalk with the nuclear components [5], suggesting that the positioning of PcG bodies in the nucleus could be highly regulated.

Another important aspect of PcG proteins is their dynamism. In fact, PcG proteins are extremely important for lineage commitment, development, and cell differentiation, when a proper timing of gene expression is needed. Upon differentiation stimuli, PcG proteins leave lineage-specific promoters and bind genes important for stemness maintenance [6]. These processes require a highly regulated, coordinated, and fast re-localization of PcG proteins inside the nucleus followed by chromatin remodeling.

Being involved in various biochemical dynamic processes and working at different chromatin levels, PcG biology has inspired several new technical approaches aimed at dissecting the complex molecular mechanisms which together determine their function. Many of these experimental approaches have provided paradigms for the study of chromatin structure and epigenetics in general.

For instance, Chromosome Conformation Capture (3C) technology [7] and its derivative technologies (reviewed in [8]), such as Chromosome Conformation Capture on chip (4C) [9] and High resolution Capture (Hi-C) [10], have been applied in the PcG-related research to shed light on the chromatin contacts occurring in the nucleus and to allow the high-throughput mapping of the genome conformation. The use of these technologies has

led to important advances in understanding PcG functions, demonstrating that the coordinated action of PcG proteins is required to form multi-looped structures where all the major PcG targets are gathered together by *cis* and *trans* interactions [11–14]. These findings were recently confirmed and corroborated by super-resolution microscopy-based studies showing that PcG protein interactions mediate the formation of a characteristic repressive chromatin folding, with a high degree of chromatin intermixing and exclusion of neighboring active chromatin [15, 16].

Hence, our knowledge concerning PcG mode of action is steadily increasing while PcG research is inspiring the development of novel technologies and the appearance of several variations on pre-existing protocols. The current special issue provides a snapshot of the most recent technologies used in the PcG field; scientists working on Polycomb have been invited to contribute with state-of-the-art detailed methods, so as to create a unique and comprehensive reference source for investigating Polycomb function in the nucleus.

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Part I

Chapter 1

Mapping the Function of Polycomb Proteins

Diego Pasini*

Abstract

Polycomb group (PcG) proteins are master regulators of proliferation and development that play essential roles in human pathologies including cancers. PcGs act as gatekeepers of cellular identity, maintaining repression of a multitude of target genes. However, these properties have only been recently uncovered thanks to technological advances, first of all chromatin immunoprecipitations (ChIP), that allowed a systematic characterization of the activity of these factors in an unbiased manner at a genome-wide level. Using PcG protein as example, this chapter introduces the readers to the use of chromatin analysis (ChIP assays and replication timing) and how to move these approaches to a level of genome-wide interpretation.

Key words Polycomb, Chromatin, ChIP, Next-generation sequencing, Replication

Polycomb group proteins (PcG) were first identified many years ago via genetic screens in *Drosophila melanogaster* as essential proteins for flies' development. Loss of PcG function resulted in spatiotemporal deregulation of homeotic genes, which result in an aberrant activation of gene expression along the anterior–posterior axis of the developing embryo. For this, PcG proteins were rapidly classified as transcriptional repressors [1, 2].

Taking advantage of *Drosophila* genetics, several laboratories were able to identify elements (PRE), often placed at long distances from promoters, at which PcG proteins were directly recruited to maintain transcriptional repression. The isolation of these genetic elements became very useful in the pre-genomic era to characterize the means by which PcG proteins are recruited to chromatin and how they repress transcription [3]. However, based on specific staining of polytene chromosomes, it also became immediately clear that PcGs were not simply bound to the few identified PREs, but likely had a much broader occupancy along the fly's genome. This prompted several laboratories to identify consensus DNA sequences within PREs that could predict PcG recruitment. However, these attempts were relatively unsuccessful and immediately suggested that the mechanisms by which PcG proteins are recruited to specific genomic loci were likely more complicated than the simple picture