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MINI-REVIEW

Polycomb repressive complex 2 in embryonic stem cells: an overview

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ABSTRACT

Polycomb Group Proteins (PcG) are a family of epigenetic regulators responsible for the repression of an array of genes important in development and cell fate specification. PcG proteins complex to form two types of epigenetic regulators: Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2). Although the mechanisms regulating PRC2 recruitment and activity in mammals remain poorly understood, recent work has identified a non-canonical PRC2 in mouse embryonic stem cells (mESC) with unique activities required for repression of PRC2 target genes and necessary for mESC differentiation and somatic cell reprogramming. Here we review the functions of PRC2 in embryonic stem cells and explore the role of the newly identified mESC specific PRC2 regulatory subunits Jarid2 (jumonji, AT rich interactive domain 2), Mtf2 (metal response element binding transcription factor 2) and esPRC2p48.

KEYWORDS polycomb repressive complex 2, histone modification, epigenetic regulation

INTRODUCTION

Embryonic stem cells must maintain a pluripotent gene expression profile while simultaneously retaining the ability to rapidly respond to signals and differentiate into any of the terminally differentiated cell types. This crucial genomic plasticity is enabled by a careful balance of traditional transcriptional regulators and newly identified epigenetic factors. Chief among the epigenetic regulators of pluripotent gene expression and cell type transition are the Polycomb Group proteins (PcG). First identified in *Drosophila* as key repressors of Hox genes during body patterning, PcG proteins have been shown to play key roles in development, X-inactivation, cell pluripotency and somatic cell reprogramming (Schwartz and Pirrotta, 2007; Kanduri et al., 2009; Schuettengruber and Cavalli, 2009; Simon and Kingston, 2009; Surface et al., 2010).

PcG proteins are known to affect their repressive functions primarily through the formation of Polycomb Repressive Complexes (PRCs). The two major PRCs, termed PRC1 and PRC2, are responsible for the posttranslational modification of histones H2A and H3. PRC1, composed of Bmi1, Ring1A, Ring1B, Cbx and Phc, is responsible for ubiquitination of histone H2A at lysine 119 (Levine et al., 2002; de Napoles et al., 2004; Wang et al., 2004a). PRC2, containing core proteins Suz12, EED (embryonic ectoderm development) and EZH1/2, catalyzes the di- and tri-methylation of histone H3 at lysine 27 (Cao et al., 2002; Czermin et al., 2002; Kuzmichev et al., 2002; Kirmizis et al., 2004). Both H2A K119 ubiquitination and H3 K27 di/tri-methylation are strongly associated with transcriptional repression. While Ring1B and EZH2 are the catalytic subunits of their complexes, initiating H2AK119ub and H3K27me3 respectively, the repressive activities of PRC1 and PRC2 require both catalytically active and inactive components and functionalities (Mateos-Langerak and Cavalli, 2008; Tiwari et al., 2008a, 2008b; Eskeland et al., 2010).

PRC1 and PRC2 mediated gene repression play key roles during embryonic development. PRC2 deficiency (whether through knockout of EED, EZH2 or Suz12) results in early embryonic lethality in mice (Faust et al., 1995; O'Carroll et al., 2001; Pasini et al., 2004). PRC1 deficiency is associated with a less severe phenotype as offsprings are in general viable but exhibit transformations and other developmental abnormalities (van der Lugt et al., 1996; del Mar Lorente et al., 2000; Akasaka et al., 2001). Only Ring1B null mutations are embryonic lethal due to a defect in gastrulation (Voncken et al., 2003; Eskeland et al., 2010). Interestingly, PRC1 and

PRC2 components do not appear to be required for embryonic stem cells (ESC) self renewal, but are necessary for differentiation and lineage commitment (Pasini et al., 2007; Chamberlain et al., 2008; van der Stoop et al., 2008).

While core components of PRC2 are highly conserved across cell types, recent work suggests that PRC2 subunit composition may vary among cell types and between developmental stages. Specifically, mouse embryonic stem cells (mESC) have been shown to contain a PRC2 complex containing at least three additional regulatory subunits which play key roles in the control of the pluripotent gene expression program and are required for cell fate transitions (Peng et al., 2009; Shen et al., 2009; Landeira et al., 2010; Li et al., 2010; Pasini et al., 2010; Walker et al., 2010; Zhang et al., in press). In this review we will discuss the role of PRC2 in cell pluripotency, differentiation and somatic cell reprogramming.

MECHANISMS OF PcG MEDIATED GENE SILENCING

Early models suggested that PcG mediated gene repression required the paired activity of both PRC2 and PRC1. In *Drosophila*, PRC2 is recruited to conserved domains termed Polycomb Response Elements (PRE) through the activity of PRE binding proteins (Chan et al., 1994; Muller and Kassis, 2006; Ringrose and Paro, 2007). PRC2 mediated H3K27me3 serves as a docking site for the PRC1 chromo-domain containing protein Cbx (crossbronx) (Fischle et al., 2003; Wang et al., 2004b). PRC1 has been shown to block transcription *in vitro*, possibly by impairing chromatin remodeling, compacting chromatin, or by inhibiting RNA polymerase II (RNAP II) elongation (Francis et al., 2004; King et al., 2005; Stock et al., 2007; Brookes and Pombo, 2009). Some recent work points to a more complex mechanism of gene repression. PRC1 and PRC2 share similar but not entirely overlapping patterns of gene occupancy, indicating a role for PRC2-independent PRC1 recruitment and gene repression (Schoeftner et al., 2006; Schwartz et al., 2006).

Mammals, which lack most PREs, have evolved several mechanisms to direct and regulate the recruitment of PRCs (Sing et al., 2009; Woo et al., 2010). YY1 binding protein and PRC1 interacting partner RYBP (RING1 and YY1 binding protein) appears to direct the recruitment of PRC1 to YY1 transcription factor binding sites (Woo et al., 2010). Long noncoding RNA's (lncRNA) may serve as scaffolds, recruiting PRC2 to specific sites (Rinn et al., 2007). PRC2 core component Suz12 has been shown to interact with the short transcripts produced from CpG islands, which are enriched upstream of many PcG target genes (Kanhere et al., 2010). These short transcripts can recruit PRC2 resulting in gene repression *in cis*. More recently, non-canonical mESC PRC2 component Jarid2 has been proposed to direct recruitment of PRC2 through its DNA binding domains (Peng et al., 2009; Shen et al., 2009; Li et al., 2010; Pasini et al., 2010).

PRC2 CONTAINS REGULATORY SUBUNITS IMPORTANT FOR EMBRYONIC STEM (ES) CELL SPECIFIC FUNCTIONS

In terminally differentiated cell types, PcG proteins are responsible for maintaining the differentiated cell phenotype through repression of all unnecessary genes. In embryonic stem cells, PcG proteins are enriched at key developmental regulators (Boyer et al., 2006; Bracken et al., 2006; Lee et al., 2006; Ku et al., 2008) and appear to stabilize the pluripotent gene expression program while potentiating differentiation (Pasini et al., 2007; Chamberlain et al., 2008; van der Stoop et al., 2008; Leeb et al., 2010). These loci are associated with increased levels of PRC2 mediated histone modification H3K27me3 and are transcriptionally repressed (Boyer et al., 2006). During ESC differentiation, PcG target genes are preferentially activated. Knockout of PRC2 components EED, Suz12 and EZH2 in mESC alters global H3K27me3 patterns but does not destroy the self renewal capabilities of these cells (Pasini et al., 2007; Chamberlain et al., 2008; Shen et al., 2008). Nevertheless, these cells show increased expression of PcG targeted developmental regulators, are prone to spontaneous differentiation, and undergo defective differentiation (Boyer et al., 2006; Pasini et al., 2007; Chamberlain et al., 2008). It is theorized that PRC2 catalyzed H3K27me3 in mESC serves first as a buffer against differentiation in response to improper or weak stimuli and second as a mark required for the establishment of differentiated cell type specific gene expression patterns (Chi and Bernstein, 2009). These unique functions of PRC2 in ESC are directed by newly identified regulatory subunits Jarid2, Mtf2 (Pcl2), and esPRC2p48.

Jarid2 (jumonji, AT rich interactive domain 2), Mtf2 (metal response element binding transcription factor 2) and esPRC2p48 are confirmed by reciprocal co-immunoprecipitation as *bona fide* subunits of PRC2 in mESC (Peng et al., 2009; Shen et al., 2009; Landeira et al., 2010; Li et al., 2010; Pasini et al., 2010; Walker et al., 2010; Zhang et al., in press). ChIP (chromatin immunoprecipitation) experiments confirm that Jarid2 distribution overlaps nearly all sites occupied by PRC2 core component EZH2, suggesting that PRC2 containing Jarid2 is the predominant form of PRC2 in mESC (Peng et al., 2009; Shen et al., 2009; Landeira et al., 2010; Li et al., 2010; Pasini et al., 2010). Jarid2, Mtf2 and EZH2 colocalize at approximately 50% of sites (Li et al., 2010). Previous research has demonstrated that EZH1 and EZH2 form mutually exclusive complexes and that PRC2 containing EZH1 possesses intrinsically lower methyltransferase activity than EZH2 containing PRC2 (Margueron et al., 2008). Research conducted in our laboratory confirms that, while EZH1 and EZH2 do not coimmunoprecipitate in mESC, both EZH1 and EHZ2 containing PRC2 are present in mESC. Interestingly, reciprocal immunoprecipitations indicate that Jarid2, Mtf2 and esPRC2p48 associate with both EZH1 and

EZH2 containing PRC2 (Shen et al., 2009; Zhang et al., in press). The precise role of the EZH1 containing mESC PRC2 has not been well studied.

Jarid2 contains an ARID DNA binding domain in addition to a catalytically inactive JmjC domain (Cloos et al., 2008). Jarid2 has been shown to both inhibit (Peng et al., 2009; Shen et al., 2009) and stimulate (Li et al., 2010; Zhang et al., in press) PRC2 methyltransferase activity *in vitro*. These contradictory results are likely due to variations in experimental protocol. Jarid2 depletion impairs but does not abolish PRC2 recruitment to target genes, pointing to the presence of alternative methods of PRC2 targeting in mESC (Peng et al., 2009; Shen et al., 2009; Landeira et al., 2010; Li et al., 2010; Pasini et al., 2010). The ARID domain is required for Jarid2 directed PRC2 recruitment (Pasini et al., 2010), but its role in direct DNA binding is unclear (Li et al., 2010). Interestingly Jarid2 binding to target genes is also impaired upon EED knockout, suggesting synergistic recruitment (Peng et al., 2009; Shen et al., 2009; Li et al., 2010).

Mtf2 is one of three mammalian homologs for the *Drosophila* protein Pcl (Polycomblike), a known PRC2 interacting protein. Mtf2 is thought to direct repression of a subset of genes known to stabilize master regulators of pluripotent gene expression including Klf2, thus regulating the robustness of the pluripotent gene expression program (Walker et al., 2010).

esPRC2p48, a previously uncharacterized protein, is predicted to be structurally unordered and may serve a scaffolding function. In *in vitro* assays, esPRC2p48 enhances the H3K27me3 activity of PRC2 individually and in a synergistic manner with Jarid2 or Jarid2 and Mtf2 but not with Mtf2 alone (Zhang et al., in press).

PRC2 REGULATORY SUBUNITS ARE REQUIRED FOR DIFFERENTIATION

The expression of these newly identified PRC2 subunits is controlled by pluripotency regulator Oct4 (Boyer et al., 2005; Loh et al., 2006; Kim et al., 2008). Upon *in vitro* differentiation, Jarid2, Mtf2 and esPRC2p48 are rapidly downregulated, implying that they execute their functions in ESC or during the early stages of differentiation (Mikkelsen et al., 2007; Walker et al., 2007; Zhou et al., 2007; Zhang et al., in press). Experimental results support roles for these subunits in both cases.

Jarid2 knockout is embryonic lethal between days E11.5 and 15.5 (Takeuchi et al., 1995). Jarid2 knockout or knockdown ESC retain self renewal properties, but undergo defective differentiation, potentially due to failure to repress Oct4 (Takeuchi et al., 1995; Shen et al., 2008; Li et al., 2010; Pasini et al., 2010). Mtf2 knockdown results in overexpression of pluripotent regulators Oct4 and Nanog thus supporting cell pluripotency and inhibiting cell response to signals of differentiation (Walker et al., 2010). Mtf2 depleted ESC also fail to differentiate properly, and display a general upregulation of pluripotency regulators (Walker et al., 2010). These data support a role for Jarid2 and Mtf2 in cell fate transitions and in fine tuning of the pluripotent gene expression program.

Jarid2 REGULATES PRC2 FUNCTION AT BIVALENT GENES

In terminally differentiated tissue types, the PRC2 catalyzed H3K27 tri-methylation mark is strongly associated with repressed genes and does not colocalize with histone methylation marks of gene activation H3K36me3 or H3K4me3. In ESC, H3K27 tri-methylation is enriched at genes associated with lineage commitment and differentiation (Boyer et al., 2006; Bracken et al., 2006; Lee et al., 2006; Ku et al., 2008). Although the repression of differentiation associated genes plays a role in the maintenance of a stable pluripotent phenotype, ESC must retain the ability to express these differentiation associated genes upon exposure to proper signals. Interestingly RNAP II and the gene activation associated mark H3K4me3 occupy a significant subgroup of H3K27 tri-methylated genes in ESC (Azuara et al., 2006; Bernstein et al., 2006; Pan et al., 2007; Zhao et al., 2007). These genes are enriched for developmental regulators and are termed *bivalent genes*. It is theorized that these bivalent genes are maintained in a poised state, allowing for prompt execution of differentiation associated gene expression programs. Upon terminal differentiation, the bivalent signature is converted either to a stable active (H3K4me3) or repressed (H3K27me3) state, presumably through the action of H3K27 or H3K4 demethylases respectively.

Newly identified mESC PRC2 regulatory subunit Jarid2 appears to be enriched at bivalent genes (Landeira et al., 2010; Li et al., 2010). Jarid2 possesses an *in vitro* methyl-protective activity, stabilizing the PRC2 catalyzed H3K27me3 mark by protecting it from the activity of H3K27 demethylases (Zhang et al., in press). While Jarid2 deficient ESC do not display global defects in H3K27me3, Jarid2 knockdown has been shown to impair H3K27me3 and is associated with reduced levels of H2AK119ub at target genes (Shen et al., 2009; Landeira et al., 2010; Li et al., 2010). Whether this is due to impaired recruitment, accelerated H3K27me3 demethylation, or diminished PRC2 methyltransferase activity is unclear. Additionally, recent work suggests that Jarid2 is crucial for establishment of the poised RNAP II posttranslational modification, a key characteristic of the bivalent gene state (Landeira et al., 2010). Despite the variable evidence, it is clear that Jarid2 plays a crucial role in cell differentiation. Further work is necessary to determine the specific role of Jarid2 at bivalent genes.

PRC2 REGULATORY SUBUNITS ARE REQUIRED FOR SOMATIC CELL REPROGRAMMING

Infection of mouse embryonic fibroblasts (MEF) with viruses encoding Oct4, Sox2, c-Myc and Klf4 results in reprogramming

of a small number of somatic cells to an ESC-like state (Takahashi and Yamanaka, 2006; Wernig et al., 2007; Park et al., 2008). The transition from somatic cell to induced pluripotent stem cell (iPS cell) involves radical alterations in the epigenetic landscape of the cell (Fig. 1). The epigenetic signature of iPS cells resembles but is not identical to the epigenetic signature of embryonic stem cells (Lagarkova et al., 2010; Ohm et al., 2010; Stadtfeld et al., 2010). For this reason, iPS cells retain an epigenetic memory of their somatic progenitors, as demonstrated by recent work showing that iPS cells are more easily differentiated into the cell type from which they were derived (Kim et al., 2010; Polo et al., 2010). Better understanding of the role of epigenetic regulators in reprogramming is a necessary step in the creation of more ESC-like iPS cells.

The pivotal roles of mESC PRC2 regulatory subunits Jarid2, Mtf2 and esPRC2p48 in regulating cell fate transitions from pluripotent to differentiated cell types are recapitulated during somatic cell reprogramming. During this transition, expression levels of mESC PRC2 regulatory subunits Jarid2, Mtf2 and esPRC2p48 increase (Zhang et al., in press). Interestingly, co-infection of MEF with a polycistronic viral vector encoding Oct4, Sox2 and Klf4 (OSK) in addition to virus encoding these regulatory subunits markedly increases the efficiency of reprogramming while knockdown of either Jarid2, Mtf2 or esPRC2p48 blocks reprogramming (Zhang et al., in press). This result is consistent with the observation that PRC2 is required for successful reprogramming (Pereira et al., 2010). The effect of Jarid2, Mtf2 and esPRC2p48 overexpression on the epigenome and differentiation potential of these iPS cells is an area requiring additional study.

CONCLUDING REMARKS

Cell fate transitions require the epigenetic reprogramming of a cell, dramatically altering patterns of gene expression to create the desired cell phenotype. ESC possess a unique epigenetic landscape, capable of maintaining the pluripotent state while remaining poised to differentiate. PRC2 is responsible for regulating gene expression through posttranslational modification of histone H3. In ESC, this functionality is fine tuned by the addition of several regulatory subunits which direct and modify PRC2 behavior.

The differentiation of progenitor cells into terminally differentiated cell types and the de-differentiation of somatic cells into progenitor cells are fundamentally epigenetic phenomena. Recent work also shows them to be intimately linked through the functions of PRC2 regulatory subunits Jarid2, Mtf2 and esPRC2p48. Transformation of normal cells into a cancerous state shows many similarities to normal cell fate transitions and somatic cell reprogramming. A deeper understanding of the detailed mechanisms by which PRC2 proteins potentiate such dramatic changes in gene expression will have great clinical significance.

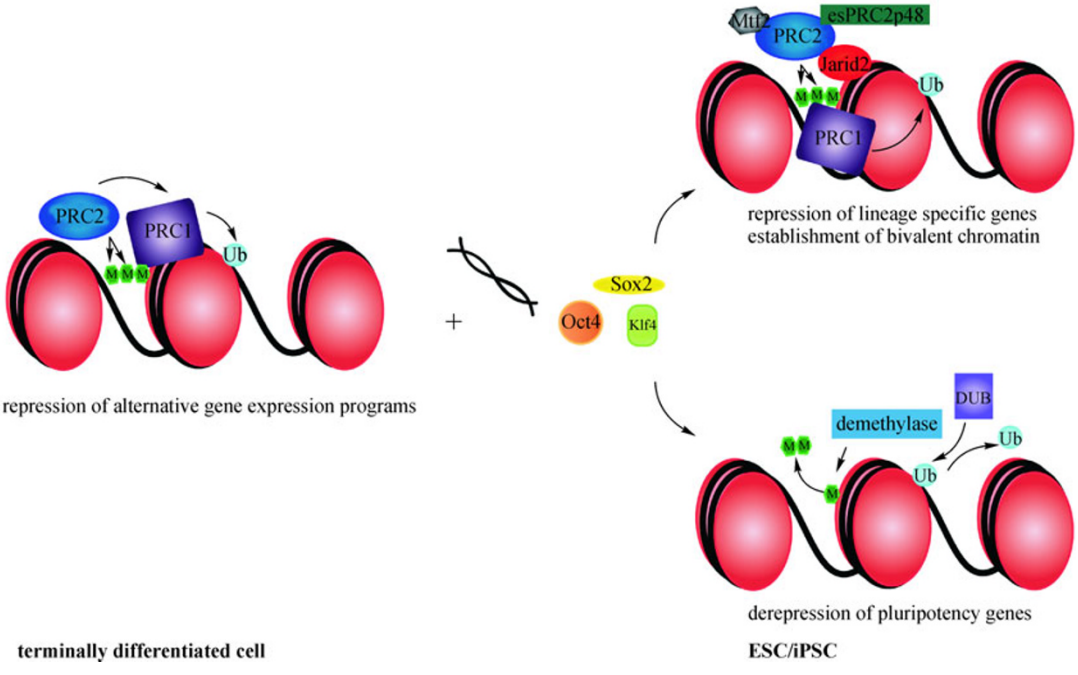

Figure 1. PRC2 regulatory subunits during cell fate transition. PRC2 represses alternative gene expression programs in terminally differentiated cells (left). During cell reprogramming, lineage specific genes are targeted for repression by PRC2 complexed with newly identified regulatory subunits (upper right) while pluripotency genes are de-repressed through the action of putative histone demethylase(s) and deubiquitinase(s) (lower right). PRC2, Polycomb Repressive Complex 2; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell.

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ABBREVIATIONS

ChIP, chromatin immunoprecipitation; EED, embryonic ectoderm development; ESC, embryonic stem cells; mESC, mouse embryonic stem cells; Jarid2, jumonji, AT rich interactive domain 2; Mtf2, metal response element binding transcription factor 2; PcG, Polycomb Group Proteins; Pcl, Polycomb like; PRC1 and PRC2, Polycomb Repressive Complex 1 and 2; PRE, Polycomb Response Elements; RNAP II, RNA polymerase II; RYBP, RING1 and YY1 binding protein

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