

# DYNAMIC STUDY OF POLYCOMB REPRESSION MEDIATED BY THE PRC1 PROTEIN COMPLEX

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## ABSTRACT:

Histone lysine methylation plays a fundamental role in chromatin organization and function. This epigenetic mark is involved in transcriptional regulation. In particular, methylation at H3K27 is associated to transcriptional silencing and gene repression. This repression state is maintained by the PRC1 complex of Polycomb Group proteins (PcG), whose accumulate in subnuclear foci known as Polycomb bodies. In mammals, the PRC1 complex is very complex due to the high number of PcG orthologs and there is evidence that different PRC1 complexes exist in the same cell. Moreover, the molecular mechanisms of PRC1-mediated repression remain unclear.

Using fluorescence microscopy and GFP-tagged proteins, we firstly showed that different CBX proteins do not colocalize in the same Polycomb bodies, indicating that different types of Polycomb bodies might exist in cell. Secondly, to gain insight into the formation mechanisms of PRC1 complex and the processes maintaining the repression, we used FRAP experiment to determine the dynamic behaviour of PRC1 proteins. We showed that all of the PRC1 proteins tested are mobile that suggesting a dynamic system to maintain the repression state in cell. In addition, protein belong to different group, like Pc (CBX), Psc (BMI1) and Ring (RING1A), displayed different dynamics, demonstrating that PRC1 complex was not preformed but one of PRC1 protein recognized the H3K27me3 and induce the recruitment of the other partners. Moreover, inside core protein (Pc) group, CBX2 and CBX4 present a different dynamic behaviour with two different residences times (22,3s for CBX2 vs. 63,7s for CBX4); CBX4 was more static than CBX2. These significant differences in dynamics within Polycomb bodies demonstrate the formation of two different PRC1 complexes, which probably repressed different genes.

In the future, fluorescence lifetime measurement (FLIM) coupled with the fõster resonance energy transfer (FRET) will be use to measure protein-protein interaction within the PcG protein assemblies at Polycomb bodies.