

Live imaging of Polycomb Repressive Complex 1 dynamics in mitotic and differentiating chromatin of *Drosophila melanogaster*

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Abstract: The Polycomb and Trithorax groups of proteins (PcG and TrxG) are responsible for the maintenance of the epigenetic memory of repressed and active transcription states of several hundred developmentally important target genes in *Drosophila* [1]. Although the mechanism of establishment of transcriptional states in some of the target genes is relatively well characterised, the mechanism underlying the maintenance of transcriptional activity from early embryo to the adult fly through mitosis is still undetermined. Interestingly, it is known that PcG exchange rapidly from the chromatin with different kinetics at different loci [2] and that locus-specific difference in stability correlate well with the transcriptional status of the associated genes [3].

To understand how the dynamic behaviour of PcG/TrxG proteins can nevertheless allow the stable maintenance of an epigenetic memory through mitosis, we have established quantitative live-imaging techniques in *Drosophila melanogaster* larval and pupal tissues to ask about the kinetic properties of PcG, TrxG and DNA binding fluorescent fused proteins during mitosis. To extend published studies describing the kinetic properties of two PcG members (Pc and Ph) in embryos, larval wing imaginal discs and salivary glands [2], we have established the same techniques on neuroblasts in the larval brain and sensory organ precursor cells (SOP). Because these cells divide asymmetrically [4] we have thus established a system in which to study PcG/TrxG properties during mitosis and at the same time during a differentiation pathway. Our initial results show different accumulation of GFP-fused Pc and Ph in mitotic chromosomes of neuroblasts and SOPs, which are not only due to changes in cell and nucleus volume, but also reflect changes in kinetic properties at the interphase-mitotic transition.

We will proceed with this analysis on other proteins of the PcG (E(Z)), TrxG (Ash1) and on DNA binding proteins (Pho and DspI) to evaluate further differences or similarities between different proteins. We will also, through photobleaching experiments, determine binding constants of these different proteins to interphase and mitotic chromatin in order to create a mathematical model of how protein dynamics contribute to the regulation of transcriptional status and maintenance of this status through mitosis.

References:

- [1] Ringrose, L and Paro, R (2007). Polycomb/Trithorax response elements and epigenetic memory of cell identity. *Development*. **134**:2, 223
- [2] Ficz, G, Heintzmann, R and Arndt-Jovin, D (2005). Polycomb group protein complexes exchange rapidly in living *Drosophila*. *Development*. **132**:17, 3963
- [3] Ringrose, L, Ehret, H and Paro, R (2004). Distinct contributions of histone H3 lysine 9 and 27 methylation to locus-specific stability of polycomb complexes. *Mol Cell*. **16**:4, 641
- [4] Betschinger, J and Knoblich, J (2004). Dare to be different: asymmetric cell division in *Drosophila*, *C. elegans* and vertebrates. *Curr Biol*. **14**:16, R674