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Identification des modifications post-traductionnelles de l'histone H3 impliquées dans la condensation des chromosomes "in embryo"

Epigenetic modifications and nuclear architecture are globally rearranged after fertilization. Unlike somatic cells, mammalian embryos for example present a unique organization of pericentromeric heterochromatin. In mouse embryos, this part of the heterochromatin is not organized in clusters but in spherical structures around nucleolar precursor bodies forming a "cartwheel". This pericentric heterochromatin is characterized by specific epigenetic marks, in particular trimethylation of histone H3 at Lysine 9 (H3K9me3), together with the heterochromatin protein 1 (HP1b).
Surprisingly, we recently found out that this heterochromatin also contains histone H3 phosphorylated at serine 10 (H3S10P) from early interphase through mitosis over several embryonic cycles (Mason et al., 2012). In somatic cells, it is known that when histone H3 is phosphorylated at S10 at the end of the cell cycle, HP1b is ejected from the chromatin upon the entry in mitosis. We therefore questioned the "colocalization"of H3S10P and HP1b we observed in early stage embryos, even in G1/S phases.
To better understand the mechanisms behind HP1b association/ejection in early embryos, we performed immunostaining with several antibodies directed against HP1b, H3K9me3, H3S10P as well as with an antibody specific to the double modification H3K9me3S10P.
In order to get a deeper insight into the colocalization between these epigenetic marks/proteins we also developed a new approach based on the in situ PLA technology (Proximity Ligation Assay). This technique allows the fluorescent detection of two targets when they are in close proximity (<40 nm) without any genetic manipulation of the cells in contrast to other techniques such as BRET or FRET.
Altogether our results demonstrate that H3S10P and H3K9me3 can both colocalize with HP1b but that only the double modification H3K9me3S10P is responsible of HP1b ejection from chromatin only upon the first mitosis in mouse embryos.

Auteur principal: Dr BEAUJEAN, Nathalie (INRA)

Orateur: Dr BEAUJEAN, Nathalie (INRA)