Link between organization and transcription of Ribosomal RNA genes in mouse growing oocytes and at the very beginning of embryonic development

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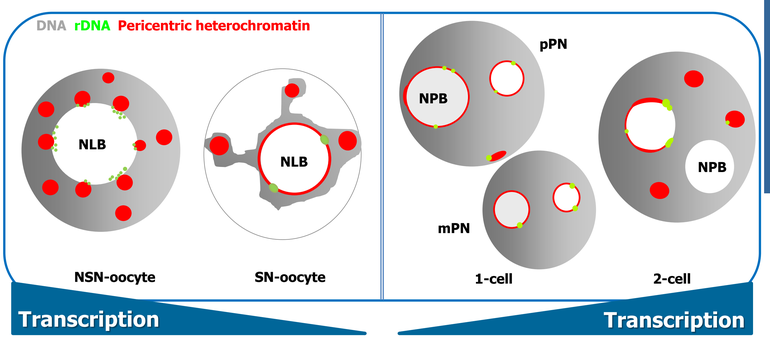

LINK BETWEEN ORGANIZATION AND TRANSCRIPTION OF rRNA GENES IN MOUSE GROWING OOCYTES AND AT THE VERY BEGINNING OF EMBRYONIC DEVELOPMENT

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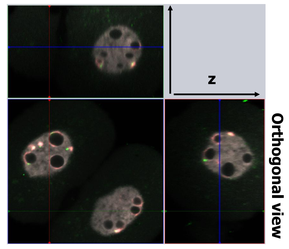
Introduction

During this final step of oogenesis, the oocyte nucleus is subject to large-scale modifications concurrently with transcriptional silencing. While oocytes with dense chromatin surrounding the nucleolus (SN) are silent, oocytes with uncondensed chromatin (non-surrounded nucleolus, NSN) are transcriptionally active (Bouniol-Baly, 1999). In a same manner, at the very beginning of the mouse development (1-cell and early 2-cell stages), transcription of ribosomal genes (rDNA) is switched off and ribosomal RNA (rRNA) synthesis starts at the end of the 2-cell stage (Zatsepina et al. 2003). Our previous studies show- that nucleolus-like structures (nucleolus like bodies - NLBs or nucleolar precursor bodies - NPBs) are associated with pericentromeric heterochromatin. Indeed, pericentromeric heterochromatin is relocated and condensed when euchromatin transcription is switch on (Aguirre-Lavin et al, 2012) or decondensed when global transcription is switch off (Bonnet-Garnier et al, 2012). In somatic cells, Guetg *et al.* (2010; 2012) have observed that silencing of rDNA contributes to the maintaining of pericentromeric heterochromatic state. Our objective is to investigate the organization of rDNA versus pericentrometric heterochromatin sequences at the light of their transcriptionnal states (active: NSN and 2-cell stage vs inactive: SN and 1-cell stage) using 3D DNA and RNA-FISH techniques (Aguirre-Lavin et al, 2012 ; Miyanari and Torres-Padilla, 2012).

Material and Methods

We studied the distribution of ribosomal DNA and pericentromeric sequences (major satellite) in relation to nucleolar bodies with specific probes using 3D DNA-FISH protocol (Bonnet-Garnier et al. 2012). The ribosomal genes transcription was revealed with a 3D RNA-FISH protocol adapted from ME T Padilla (date) using 5'-ETS, ITS2 and 18S specific oligonucleotids (20-30nt) coupled in 5' with a fluorescent –dye (alexa 488, Cy3 or Cy5)



Images were acquired with a Zeiss LSM700 confocal microscope (MIMA2 facilities) and 20 to 70 nuclei were analysed per stages.

Results

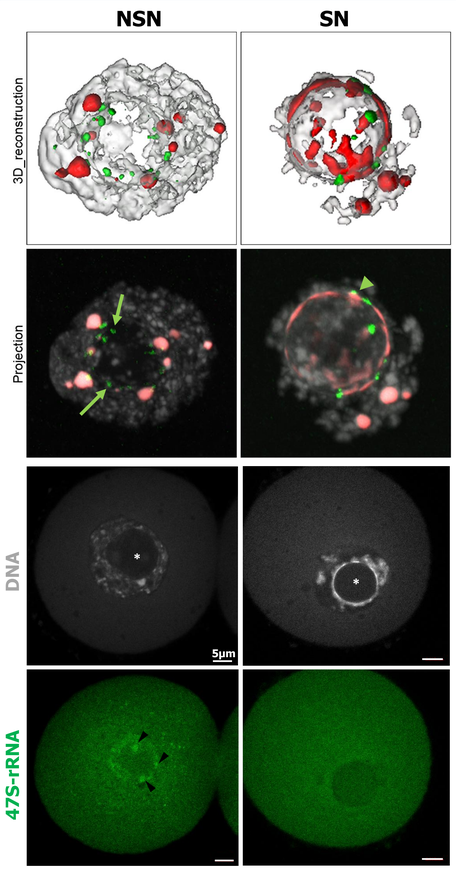
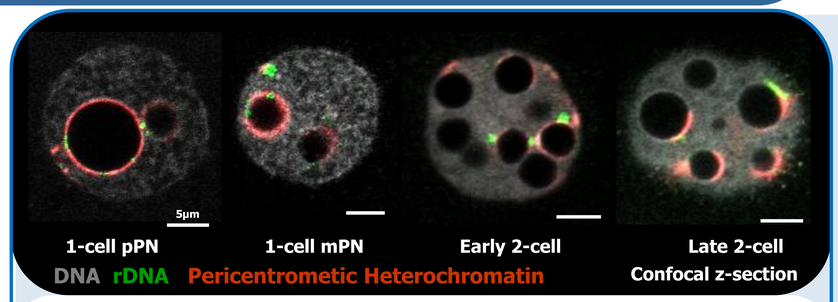


Figure 1

In NSN-oocytes, rDNA sequences are decondensed as illustrated by their pearl necklace structure (green arrows, Fig. 1), transcribed (revealed by RNA-FISH, black arrow head) and not associated to pericentromeric heterochromatin; while in SN-oocytes, they gather together in seven highly condensed foci at the NLB periphery tightly associated to pericentromeric sequences (green arrow head) and are not transcribed.

At 1-cell and early 2-cell stages, no signal were detected when using probes targeting ITS2 and 28S rRNA (Fig. 3) confirming that no transcription or processing of rRNA occurred at these stages. At the late 2-cell stage, rRNA genes start to transcribed and pre-rRNA are processed (Fig. 3).



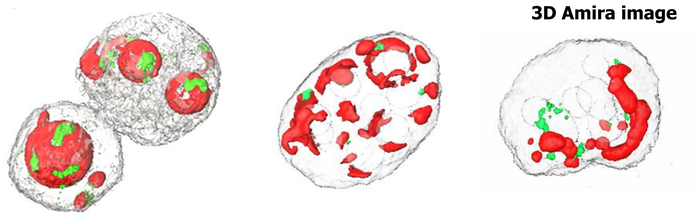


Figure 2 1-cell Early 2-cell Late 2-cell

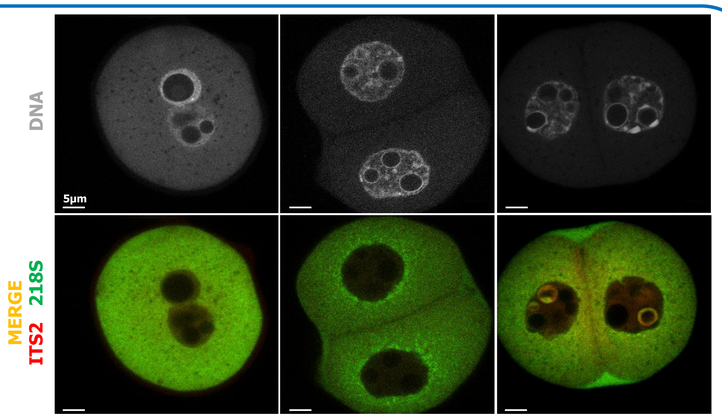


Figure 3 1-cell Early 2-cell Late 2-cell

At 1-cell and early 2-cell stages, the ribosomal sequences are clustered and juxtaposed to pericentromeric sequences forming a discontinuous ring around NPBs. the total number of rDNA signals observed by FISH began to increase at the late 2-cell stage (Fig. 2 and 4) and increases dramatically from the 4-cell to the 16-celll stages (data not shown). Concomitantly with the relocation and the condensation of pericentromeric heterochromatin in chromocenter, the number of rDNA signal associated to major satellite signal decreased (Fig. 4).

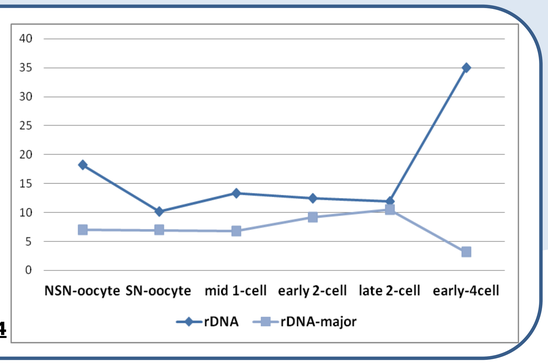


Figure 4

Conclusion

Ribosomal genes are organized in compact clusters associated to decondensed pericentromeric sequences when completely silent in SN-oocytes and at the 1-cell and early 2-cell stages. Conversely they are decondensed and less linked with compacted pericentromeric regions when their transcription is massively switch on in NSN-oocytes, at the 2-cell and 4-cell stages. So, depending on their transcription state, ribosomal sequences are more or less in contact with pericentromeric regions. Next, we could question how rDNA and pericentromeric repeat sequences interplay to regulate their heterochromatic state.

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