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Meiotic silencing of unsynapsed chromatin: localisation of the phosphorylated histone γH2A.X in spermatocytes of homozygous and Robertsonian heterozygous mice

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The aim of my research work was to investigate the Meiotic Silencing of Unsynapsed Chromatin (MSUC), a peculiar aspect of male mouse meiosis, in a natural model animal of spermatogenic impairment, giving particular attention to XY silencing, association of autosomes with the XY body, positivity to γH2A.X of the trivalents and their degree of asynapsis[1]. The temporal appearance and disappearance of γH2A.X during Prophase I was also monitored.
For this purpose, heterozygous mice were obtained by breeding homozygous female of the CD1 strain (2n=40 all acrocentric chromosomes) with homozygous male of the Milano II Robertsonian (Rb) population (2n=24 acrocentric and metacentric chromosomes). Homozygous CD1 and Milano II males were also used to compare the dynamic of the meiotic pairing and γH2A.X appearance and localisation. Three, five and seven months old mice were used. At the prophase stage of meiosis I, heterozygous males show characteristic figures of the paired autosomes called trivalents. These trivalents had different degree of asynapsis.
The analysis of the presence of phosphorylated γH2A.X showed that this modified histone was present of the XY body nearly in 100% of the spermatocytes, as expected, of both homozygous and heterozygous mice. γH2A.X signals were found also in autosomes associated with the sex body. The frequency of association was higher in Rb heterozygotes (about 45%) compared to Rb homozygotes (20%) or acrocentric homozygotes (less than 10%). γH2A.X signals were present on unsynapsed regions of trivalents. From these results it emerges that it is asynapsis of the chromatin regions per se that induces the silencing cascade and not the vicinity of the autosomes to the sex body. In fact, γH2A.X positive signals were present in those chromosomal regions that showed asynapsis independently of the proximity of the autosomes to the XY body. The results of my research work confirm the presence of a mechanism controlling the unsynapsed region of chromatin not restricted to the unsynapsed regions of the sex chromosomes. However, spermatocytes with unsynapsed regions are able to progress from prophase to metaphase. The analysis of apoptosis in the testis of Robertsonian mice[2] has shown that the percentage of TUNEL positive tubule cross sections was significantly higher in testis of Rb heterozygous than that in either CD1 or Mil II mice; infact, Rb heterozygotes showed a tenfold increase. Most of apoptotic cells in Rb heterozygous testis has been observed in tubules at stage XII where spermatocytes undergo meiotic divisions. In particular, TUNEL positive cells were spermatocytes at metaphase I an II indicating that they were dying by apoptotic processes.

Developmental competence of antral oocytes obtained by nuclear transfer

During the last four months, I was working to a new research project aimed to study the molecular determinants of the 2-cell block during the development of mouse embryos active in some strains. For this purpose, two different mouse strains (CD1, that presents the 2-cell block, and B6C3F1, that does not) were used. In order to understand if the 2-cell block is determined by a cytoplasmic inheritance or is determined by the nucleus of the egg, the metaphases II of oocytes of the CD1 strain were transferred to enucleated cytoplasm of oocytes of the B6C3F1 strain. The reciprocal transfer was also performed. The reconstituted oocytes were then fertilised *in vitro*. The expression of early development specific genes, like *Oct4, Zar1, Mater, Prei3, Smarca4, Stella* and *Nucleoplasmin 2,* will be analysed in embryos obtained by MII transfer and will be compared with control embryos.

Publications

1. Merico V., Diaz de Barboza G., **Vasco C.**, Ponce R., Rodriguez V., Garagna S. and Tolosa de Telamoni N. (2008). *A mitochondrial mechanism is involved in apoptosis of Robertsonian mouse germ cells. Reproduction* (2008) 135: 797–804

2. Meiotic silencing of unsynapsed chromatin: localisation of the phosphorylated histone γH2A.X in spermatocytes of homozygous and Robertsonian heterozygous mice". **Work in progress**.