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CHROMOSOME TERRITORIES IN MICE SPERMATOGENESIS: A NEW THREE-DIMENSIONAL METHODOLOGY

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Chromosomes occupy specific nuclear regions called chromosome territories (CT) which are arranged in cell-type specific non-random patterns and are involved in genome regulation. In spermatogenic cells, a non-random distribution of chromosomes has been demonstrated in pachytene and metaphase I spermatocytes and in spermatozoa. However, most studies have been carried out using two-dimensional strategies, in particular spermatogenic stages and evaluating few chromosomes.

To overcome these limitations, we have developed an *in situ* fluorescent hybridization (FISH)-based protocol to approach the three-dimensional study of CTs along spermatogenesis. Testicular tissue from fertile mice C57BL6/6J was enzymatically disaggregated. To preserve the nuclear structure, cell suspensions were spread out on polylysine-coated slides and fixed with paraformaldehyde. Subsequently, slides were frozen in liquid nitrogen and treated with pepsin. Three successive FISH rounds were carried out using the customized kit Chromoprobe Multiprobe® OctoChrome™ Murine System (Cytocell Ltd, Cambridge, UK) designed to identify the entire mouse karyotype. This kit uses seven different combinations of three whole chromosome painting probes directly labeled in three different fluorochromes. Afterwards, SYCP3 and H1T were identified by immunofluorescence staining to categorize among pre-meiotic cells, meiotic figures (discriminating all stages from leptotene to metaphase I and metaphase II), post-meiotic cells and spermatozoa. Serial optical sections of all cell types were obtained with a *TCS-SP5* confocal microscope coupled to an imaging analysis system (*LAS AF-1.8.1*). After processing images by *ImageJ*, *Matlab* developed scripts were used to align, normalize and process nuclei in order to determine chromosome volume and proportion, chromosome radial position and chromosome relative position.

The application of the methodology developed allows the establishment of CTs throughout all spermatogenic stages providing a new basis to study the relationship between chromosome positioning and genome regulation.

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Key words: Chromosome territories, chromosome painting probes, spermatogenesis, three-dimensional analysis