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Super-Resolution Analysis of Bisphenol A Effects on Meiotic Recombination in spermatocytes

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A key step in sexual reproduction occurs during meiosis and results in recombination of the maternal and paternal chromosomes to create unique genetic combinations within the produced gametes. This mixing of the genetic material occurs through meiotic recombination (MR), a meiosis specific process during which homologous chromosomes undergo synapsis, and DNA sequence exchange, through the formation and resolution of DNA double-strand breaks. Although many key proteins and steps involved in MR have been characterised, our molecular level understanding remains limited because we previously lacked the technology to visualise these processes. Single molecule localisation super-resolution microscopy (SRM) entails single molecule sensitivity and allows for the visualisation of cellular architectures at 10 to 20 nm spatial resolutions. Recently, Zwettler *et. al*, employed SRM to reveal the nanostructure of the synaptonemal complex which bridges the homologous chromosomes during MR (1). Building on this, we aimed to visualise the spatiotemporal progression of MR by visualising and interrogating key recombination proteins including SYCP3, γH2AX, MLH1, and RAD51 during meiosis. SYCP3 identifies the lateral element protein of the synaptonemal complex (SC) whileγH2AX is a histone phosphorylation marker of DSBs. It signals for repair protein such as RAD51, which initiates DNA strand invasion to form a double holiday junction (DHJ). MLH1 follows, cleaving the DHJ to resolve and separate the homolog strands that have undergone crossover. To capture snapshots of meiosis, asynchronously growing spermatocytes were extracted from euthanised neonatal mice and spread in a monolayer on glass slides prior to fixation. Proteins were indirectly immunolabelled with Alexa Fluor tagged secondaries and SRM performed on a homebuilt microscope. As recently reported (1), SRM of SYCP3 revealed the double-helix structure of the lateral elements of the SC, which is unable to be detected using conventional fluorescence imaging. Imaging of γH2AX showed localisation to all chromosomes in zygotene prior to recombination while, RAD51 and γH2AX identified significant colocalisation with the XY chromosomes during the pachytene stage where recombination occurs.

Ultimately, the ability to clearly visualise meiotic recombination will allow for an improved understanding of the effects of toxins on these key genetic events. One such toxin is Bisphenol A, a known endocrine disruptor used ubiquitously in manufacture of plastics and many commercial products used by humans daily. Due to its structural similarity with oestrogen, BPA can disrupt endocrine and cell functions in many target organs. Why meiotic recombination levels are reduced following BPA exposure has yet to be determined, partly due to prior research into BPA's effects on reproductive cells relying on biochemical techniques and diffraction-limited florescence imaging. This study employs an advanced imaging technique known as *d*STORM super-resolution microscopy (SRM) which uses the single molecule localisation approach to map cellular structures at 10 to 20 nm spatial resolution, a 10-fold resolution increase in comparison to conventional fluorescence imaging. This enables the study of cellular processes at nanometre scales previously unseen and can be useful in investigating meiotic recombination on a molecular level.

1. Zwettler FU, Spindler M-C, Reinhard S, Klein T, Kurz A, Benavente R, et al. Tracking down the molecular architecture of the synaptonemal complex by expansion microscopy. Nature Communications. 2020;11(1):3222.