Detecting Self-associated ORC4 in Live Murine Oocytes During Polar Body Extrusion Using FLIM of eGFP and FlAsH-EDT2 Labelling

Nguyen, Brandon (School: Henry J. Kaiser High School)

In-vitro fertilization has been linked with a small but statistically significantly increased risk of mental retardation. With a such rapidly expanding field, that slight increased risk results in many children born through IVF possessing various genetic and phenotypic defects. The purpose of tracking ORC4 using fluorescent-lifetime imaging microscopy is to begin identifying a possible cause, stemming from the process by which polar bodies are extruded. ORC4 is part of the protein complex ORC, or origin recognition complex. The ORC consists of 6 protein subunits, and ORC4 is the fourth. ORC binds to the origin of replication in a dividing strand of DNA, initiating replication. During the process of female mammalian meiosis, a developing oocyte undergoes two meiotic divisions, halving its chromosome content twice, to a haploid state (n). The results of the meiotic divisions are one large oocyte, and two sets of extruded chromosomes, known as the polar bodies. Fluorescence lifetime imaging microscopy works off the difference in energy between a particle in its ground state and excited state. By exciting a molecule and allowing it to gradually lose energy, fluorescence is emitted. Live embryo protocols allowed for imaging of ORC4 movement and self-association, in which the ORC4 proteins bind to each other to form the polar body ‘cage’. The fluorescent molecules green fluorescent protein, or GFP, and fluorescein arsenical hairpin binder-ethanedithiol, or FlAsH-EDT2 were used in this experiment. The usage of the fluorescent molecules allowed for tagging and imaging of localized ORC4 proteins around the polar bodies during anaphase and G1 phase.

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