Spatio-temporal Requirements of Aurora kinase A in Meiotic Spindle Building

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Chromosome number alteration is the main determinant of early miscarriage in humans. Most errors occur during meiosis I chromosome segregation in oocytes. The Aurora protein kinases (AURK) are critical for spindle formation and chromosome alignment in both mitosis and meiosis. This family has three members: AURKA, AURKB and AURKC, of which oocytes express all three. The role of the Aurora kinases during oocyte meiotic maturation is still elusive. Previous work in our lab demonstrated that AURKA is essential for oocyte meiosis; females lacking AURKA are sterile and oocytes arrest in meiosis I with abnormal meiotic spindles. We aim to understand the requirements AURKA during meiotic maturation with a focus on temporally resolving the steps of spindle formation. In mouse oocytes, AURKA compensates for the lack of AURKB and AURKC. First, we evaluated other compensatory mechanisms that exist by creating a series of double knockout (KO) mice and using a triple KO as a negative control. We found that AURKB and AURKC can never compensate for AURKA loss because in every *Aurka* knockout background females were infertile and oocytes arrested in meiosis I. Interestingly, females lacking *Aurkb/c* but that were heterozygous for *Aurka* were fertile, and oocytes completed meiosis I and produced normal eggs. Therefore, AURKA alone is sufficient to support oocyte meiosis in mice. Next, we determined the functions of AURKA during meiotic maturation were with temporal resolution and which localized population of AURKA executes these functions. Oocytes deficient for AURKA have defects in spindle formation because they cannot fragment microtubule organizer centers (MTOC). This function is one of the first steps of spindle formation. Whether AURKA is required for later meiotic events is not known. To determine when AURK activity is needed during meiotic maturation, we used an AURKA specific inhibitor, MLN8237. We treated oocytes from AURKB/C double KO oocytes where AURKA is the only Aurora kinase present, and we compared these phenotypes to WT oocytes treated with MLN8237 to eliminate compensatory functions. We observed that AURKA activity is needed at each step of spindle building: first for MTOC fragmentation, later for MTOC fragment sorting and formation of the liquid-like spindle domain (LISD) and finally to maintain spindle stability. We asked which AURKA localized population, MTOC or chromosomes, is required for these functions. To answer this question, we ectopically expressed AURKA-CDK5RAP-GFP (MTOC targeted) or AURKA-H2B-GFP (Chromosome targeted) in triple KO oocytes. We find that neither AURKA-targeted population fully rescues spindle defects, suggesting that both populations are required, that there is a third population, or that AURKA is diffusible. Further studies are needed to distinguish between these two possibilities. These results show that AURKA is necessary and sufficient for oocyte meiotic maturation, and that it functions in multiple steps of spindle building.