Abstract

A specific feature of mammalian oocytes is a long prophase I arrest, which can be maintained for many years in humans. The oocytes must ensure robust mechanisms, which can keep them in prophase I, but effectively trigger meiotic resumption when required. Consequently, throughout the maturation of an oocyte, non-erroneous chromosome segregation is a prerequisite for the generation of healthy offspring.

In this study we aimed to investigate the new roles of Aurora A (AURKA) and polo-like kinase 1 (PLK1) in the regulation of the cell cycle progression. For this purpose, we used transgenic mice that specifically overexpress wild type (WT-) or kinase-dead (KD-) AURKA in oocytes only, and to study PLK1 we treated oocytes with BI2536, a small molecule inhibitor known to specifically inhibit PLK1 in somatic cells. Our data show, that both AURKA and PLK1 are not essential for meiotic resumption, however they participate in this process. Active AURKA regualtes the increase in microtubule organizing centers (MTOC) in prophase I, which is the first visible marker of resumption of meiosis in oocytes. AURKA activation is biphasic, and the initial increase in MTOC is transient, while full AURKA activation needed for the stability of MTOC requires the activity of Cyclin-dependent kinase 1 (CDK1). We show that PLK1 participates in meiotic resumption by promoting nuclear envelope breakdown. Both AURKA and PLK1 are needed to recruit centrosomal proteins to MTOC in order to promote normal spindle formation. In metaphase I (MI) PLK1 is needed for stable kinetochore-microtubule attachment, and inhibition of PLK1 leads to MI arrest with misaligned chromosomes activating the spindle assembly checkpoint (SAC). We show that PLK1 is required for the full activation of the anaphase promoting complex/cyclosome (APC/C) and is therefore essential for entry into anaphase I. In contrast, overexpression of WT- or KD- AURKA does not interfere with oocyte maturation.

During prophase I arrest and maturation the oocytes may be repeatedly exposed to drugs which induce DNA damage. Alternatively, endogenous DNA lesions may arise from cell's own metabolism (oxygen radicals, DNA replication and trascription), and their timely repair may increase the survival of oocytes and prevent genetic abnormalities in the embryos. We studied the response of oocytes to endogenous DNA double strand breaks (DSBs), and to DSBs induced by low concentration of the radiomimetic drug Neocarzinostatin (NCS). We found that low levels of DSBs induced by NCS in prophase I increase the incidence of chromosome fragments and lagging chromosomes but do not lead to APC/C activation and anaphase onset delay. The number of DSBs, represented by γH2AX foci, significantly decreases between prophase I and metaphase II in both control and NCS-treated oocytes. Besides, prolonged incubation of oocytes arrested in prophase I decreases the number of NCS-induced DSBs. Meiotic recombination 11 homologue (MRE11), but not Ataxia telangiectasia mutated (ATM), is essential for DSBs detection in prophase I and is involved in H2AX phosphorylation during MI. Inhibiting MRE11 by mirin during meiotic maturation results in anaphase bridges and also increases the number of γH2AX foci in metaphase II. Compromised DNA integrity in mirin-treated oocytes indicates a role for MRE11 in the DNA repair during meiotic maturation. Collectively, our data suggest that DNA repair occurs in prophase I arrested oocytes and after resumption of meiosis.