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Abstract

Cell division is a fundamental process required for the propagation of life. It takes place either by mitosis (equational genome segregation) or meiosis (reductional genome segregation). This study deals with two important players of the genome segregation machinery, kinetochore and cohesin, using *Saccharomyces cerevisiae* as a model system.

Kinetochore is a multiprotein complex required for connecting centromeres to the microtubules for the segregation of the chromosomes. Any defect in kinetochore assembly results in weak connections between centromere and microtubule, leading to the missegregation of chromosomes. In budding yeast, kinetochore is made up of around 100 proteins, categorized into eight complexes, which are further categorized as inner, central and outer complexes based on their relative position on the centromere-microtubule interface. Ctf19 supercomplex, one of the central kinetochore complexes, is made up of biochemically distinguishable three subcomplexes: COMA, Iml3-Chl4, and Ctf3. However, the functional significance of each of these subcomplexes of Ctf19 supercomplex is not known. In the first objective of this thesis, the main goal is to identify the significance of the COMA and the Iml3-Chl4 subcomplexes in meiosis. Systematic chromosome segregation analysis and meiotic progression analysis in the wild-type and the mutants of the COMA and the Iml3-Chl4 subcomplexes revealed their functional significance during meiosis, although they were found to be redundant during mitosis. The COMA subcomplex is epistatic to the Iml3-Chl4 subcomplex because absence of the COMA subcomplex proteins generates severe defects in comparison with *iml3*Δ or *chl4*Δ. Interestingly, meiotic progression analysis demonstrated that Ctf19 complex mutants arrest transiently during prophase I and metaphase I, indicating their importance during these stages of meiosis. Therefore, we aimed to observe the role of Ctf19 in executing different events of meiosis I (MI).

In the second objective of this thesis, effects of absence of Ctf19 in various MI specific events were observed. Cell biological and biochemical analysis suggested impairment of many MI specific events, such as pairing of homologous chromosomes, bi-orientation of homologous chromosomes, mono-orientation of sister kinetochores, chiasmata resolution, sister chromatid cohesion and chromosome condensation, in absence of Ctf19.

As the absence of Ctf19 affects meiosis severely than mitosis, we asked the question whether the absence of Ctf19 affects the kinetochore organization severely

during meiosis in comparison with mitosis. In the third objective of this thesis, live cell imaging and chromatin immunoprecipitation based experiments suggest that the faithful organization of the meiotic kinetochore depends more on Ctf19 in comparison with the mitotic kinetochore, as absence of Ctf19 failed to recruit many complexes at the centromere during meiosis, but not in mitosis.

The fourth objective of this thesis deals with the cohesin biology to reveal the mechanism of stepwise loss of cohesin during meiosis. Preliminary cell biological analysis has been carried out to find out the interdependencies among Sgo1, PP2A and Spo13, key protectors of the centromeric cohesin, for their involvement in the protection of centromeric cohesin during MI. Our data suggests that Spo13 and Sgo1/PP2A act through independent pathways for the protection of centromeric cohesin. Although Sgo1 and PP2A have been shown to be involved in the same pathway for the protection of centromeric cohesin during meiosis I, *sgo1*Δ and *rts1*Δ (PP2A mutant) mutants do not show similar quantity of cohesin defect, suggesting that PP2A is involved in some but not all the functions of Sgo1 for the protection of centromeric cohesin. Additionally, there are several hypotheses regarding the mechanism of deprotection of centromeric cohesin during meiosis II (MII). Here, an attempt was made to test one such hypothesis, whether increased distance between cohesin (Rec8) and its protector (Sgo1) during MII is required for the deprotection of centromeric cohesin. We designed FRET-based analysis to observe the proximity between cohesin and its protector (Sgo1) during MI and MII, but due to limitations of the FRET approach, it failed to provide conclusive information.

In summary, this work provides significant insight into the essential process of life, i.e. cell division. It increases the basic understanding of meiosis, the process required for the generation of sperm and egg cells in the human, which has a significant impact on human fertility and public health.