IITB-Monash Research Academy

Indian Institute of Technology Bombay | MONASH University

An Indian-Australian research partnership

Project Title:	**Investigation of qualitative and quantitative composition of kinetochore complex in mitosis and meiosis using yeast as eukaryotic model**	
Project Number	**IMURA0907**	
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Research Clusters: Research Themes:

Highlight which of the Academy's CLUSTERS this project will address? *(Please nominate JUST **one.** For more information, see www.iitbmonash.org)*		**Highlight which of the Academy's Theme(s) this project will address?** *(Feel free to nominate more than one. For more information, see www.iitbmonash.org)*	
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8	*HSS, Design, Management*	8	Design

The research problem

Kinetochore is a supramolecular, multi-protein complex formed at the centromere of each chromosome and connects chromosomes with that of the spindle apparatus. The complex is formed by hierarchical organization of proteins in layers (inner, central and outer) on the centromeric DNA. The approximate height of the kinetochore as resolved by EM appears to be 125 nm (Gonen et al., 2012). However, based on super resolution microscopic analysis the distance from Cse4 (centromere binding histone) to the outermost protein (Ndc80) is estimated to be 70 nm in mitotic metaphase (Cieslinski et al, 2014). It is tempting to speculate that the organization of the kinetochore proteins, both qualitatively and quantitatively, may differ between mitosis and meiosis. This organizational difference may play pivotal role in exhibiting two distinct modes of chromosome segregation. The present understanding on biology of kinetochore has stemmed mostly from study of mitotic cells. Furthermore, studies using mitotic cells have demonstrated that within a kinetochore the proteins are assembled in a hierarchical fashion (De Wulf et al, 2003). In our previous studies, analysis of requirements of several kinetochore proteins suggest that while most of the central kinetochore components are dispensable for mitotic kinetochore individually or even in combinations, absence of a single such protein can cause a drastic reduction in meiotic fitness. Interestingly, we found that the same hierarchical organization of the kinetochore proteins observed in mitosis is not followed in meiosis (Agarwal et al, 2015, Mehta et al, 2014). Overall, from our earlier studies this can be concluded that the proteome of a kinetochore between mitosis and meiosis may vary. This variation may be subjected to a change in protein composition and/or protein stoichiometry. Therefore, in this study we propose to do a comparative analysis of the proteomes of the mitotic and the meiotic kinetochores following affinity-based enrichment of the kinetochore proteins from the meiotic and the mitotic cells.

Project aims

1) Construction of isogenic yeast strains harbouring affinity tags fused to suitable kinetochore protein, capable of arresting within the cell cycle in mitosis or meiosis
2) Purification and analysis of the composition of the kinetochores from mitotic and meiotic arrested cells using high-resolution tandem mass spectrometry (LC-MS/MS).
3) Validation of the obtained differences (in kinetochore composition) in the context of mitosis or meiosis.

Expected outcomes

An error in the proper segregation of chromosomes during mitosis and meiosis leads to dire consequences like genomic instability. In human, errors in these cell division process lead to several types of diseases including cancer and also occurrence of spontaneous abortion, still birth etc (Rajagopalan and Lengauer, 2004, Hassold et al, 2007, Lengauer et al, 1997, Sen, 2000, Kops et al, 2005). Improper mitosis predisposes cells to neoplastic transformation and tumorigenesis. On the other hand, faulty meiosis leads to developmental diseases, sterility etc. Therefore, understanding the key mechanistic differences between mitosis and meiosis is very much essential to address the reasons when these two processes fail lading to diseases. However, currently there is no literature available where a direct comparison has between the mitotic and the meiotic cells taking them from the same stage of the cell cycle where these two cell cycle differs maximally. This proposed study will fill up this lacuna. Additionally, as the overall process of mitotic and meiotic cell divisions are evolutionarily conserved across eukaryotic species, using budding yeast as a model system is much more convenient than to use human model where procurement of materials is difficult for different moral and ethical reasons. The outcome of this study will not only put further thrust in overall understanding the regulation of mitosis and meiosis, but also help to understand the kinetochore biology in more detail. This will reveal how a supra-molecular structure can cater cellular multitasking through change in composition and organisation of its constituent proteins. The results will be published in peer reviewed journals.

How will the project address the Goals of the above Themes?

This is a project on basic science. However, the project requires engineering of the cells for the purpose of their arrest within the cell cycle. The cells will also be modified using molecular biology techniques to express kinetochore protein fused to the affinity tag for the purpose of purification. Therefore, the project comes under the theme of Biotechnology.

Capabilities and Degrees Required

Knowledge in Molecular biology, Microbiology and Biochemistry is required. MSc or MTech degrees are required either in Biotechnology, Microbiology, Biochemistry, Genetics or life sciences.

Select up to **(4)** keywords from the Academy's approved keyword list **(available at http://www.iitbmonash.org/becoming-a-research-supervisor/)** relating to this project to make it easier for the students to apply.

Biosciences and Biochemistry