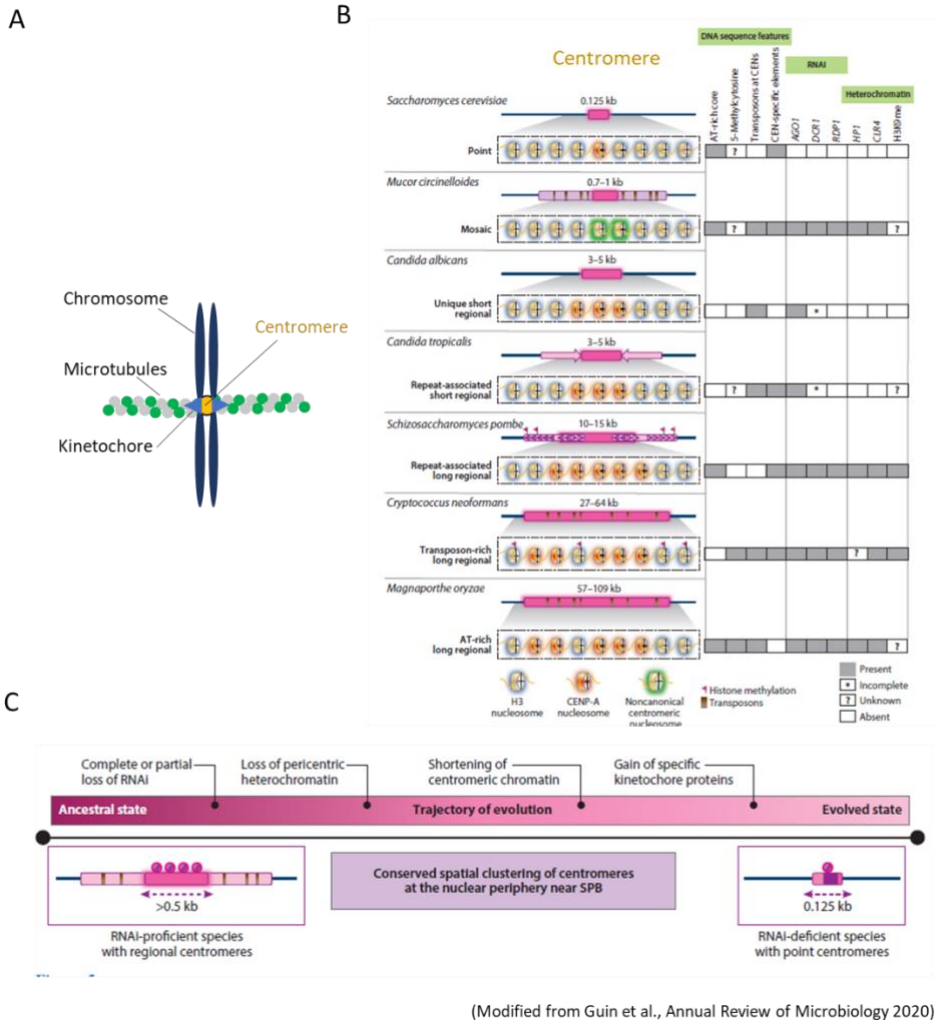
Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations.

The emerging threat of fungal infections of plants, animals and man has been highlighted since the turn of this century (Fisher et al., Nature, 2012). The estimated fatalities due to fungal infections in humans are around 1.5 million per year (Brown et al., Sci Transl Med, 2012). However, fungal infections were not appreciated by most of the scientific community, and authorities till the outbreak of *Candida auris* infection across the globe (Chakrabarti and Singh, Expert Rev Anti Infect Ther, 2020). More recently, a sudden spur in mucormycosis infection in covid19-infected patients in India sent another strong reminder to the medical community on how devastating a fungal infection can be and how ill-prepared we are in dealing with such infections. Prof. Sanyal anticipated the emergence of fungal disease in early of his career and devoted himself to this neglected field. The scientific community is facing many challenges in fungal infections due to insensitive diagnostic tools, understanding pathogenesis and developing antifungal drugs, as both host and fungi are eukaryotic and there is little scope to exploit the difference in metabolism. Unfortunately, most medically relevant fungal pathogens are genetically intractable and thus poorly studied at the molecular level. Prof. Sanyal thought of innovative approaches to tackle the problem by targeting the cell division on many pathogenic fungi, which no one earlier targeted. He collaborated with clinicians and basic scientists of various disciplines and combined molecular, biochemical, and cell biology as well as computational techniques to decipher meaningful insights into the basic biological process of chromosome segregation in fungi. His work impacts various aspects of human fungal infections, host-pathogen interactions as well as antifungal drug resistance. Identification of fungus-specific factors may help in the diagnosis and treatment of fungal infections. The key findings of his work and their impact on his own field genome stability are as follows:

Rapid evolution of centromere DNA and its implication in speciation

Centromeres act as sites for the assembly of a macro-molecular protein complex called the kinetochore. Kinetochores serve as the binding platform for spindle microtubules that power chromosome segregation during cell division. By functionally mapping centromere DNA sequences of several closely related and distantly related fungal pathogens from three different major fungal phyla, the nominee's group demonstrated that despite being involved in a conserved process of chromosome segregation, fungal centromere DNA sequences are rapidly evolving (Sanyal et al., PNAS 2004, Padmanabhan et al., PNAS 2008, Chatterjee et al., PLOS Genetics 2016, Yadav et al., PNAS 2018, Yadav et al., mBio 2019, Navarro-Mendoza et al., Current Biology 2019, Sankaranarayan et al., eLife 2020, Narayanan et al., mBio 2021). His work also demonstrates a high diversity of centromere DNA length and arrangement of sequence elements across species. This observation helped establish that centromeres are species-specific and the rapid evolution of centromeres may act as a driving force for

speciation, especially in primarily clonally propagated species (Guin et al., Annual Review of Microbiology, 2020). He discovered the existence of various types of centromeres including a novel type in an early diverging fungus, *Mucor circinelloides*, which lost the evolutionarily conserved centromeric histone CENP-A (Navarro-Mendosa et al., Current Biology 2019).

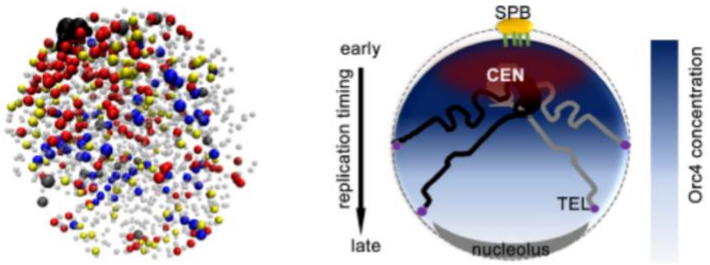


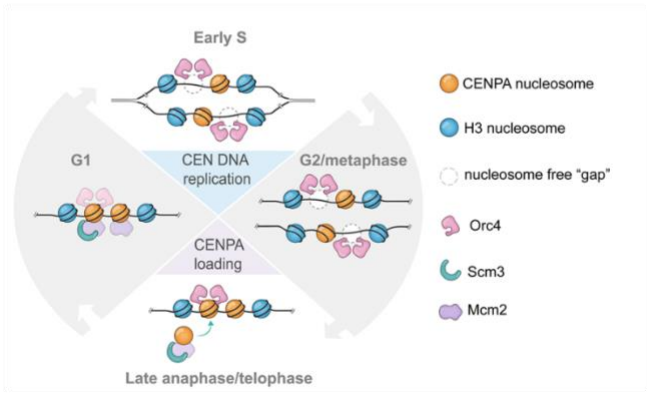
(Modified from Guin et al., Annual Review of Microbiology 2020)

Figure 1. Evolutionary trajectory of fungal centromeres. (A) Schematic showing attachment of sister chromatids to microtubules at the centromeres. (B) Diversity in fungal centromere structure and their defining features. (C) A proposed structure for the centromere of the last common ancestor for fungi.

Epigenetic regulation in centromere function

Centromere DNA sequences are not conserved across all the chromosomes in *Candida albicans* (Sanyal et al., PNAS, 2004). By elegant genetic analysis, his group activated neocentromeres by deleting the native centromere in *C. albicans*. Mapping of neocentromeres near the native centromere revealed that genomic location rather than DNA sequence is important for centromere specification (Thakur & Sanyal, Genome Research, 2013). The nominee's group also demonstrated that non-DNA sequence factors such as nearby replication origins, recombination-repair proteins, and chromosomal scaffold contribute to the centromere function in *C. albicans* (Thakur & Sanyal, PLOS Genetics, 2102; Mitra et al., PLOS Genetics, 2014; Sreekumar et al., Genetics, 2019; Sreekumar et al., Genome Research, 2021). On the other hand, studies in *Cryptococcus* species revealed that RNAi stabilizes transposons at the centromeres to maintain long repetitive centromeres and are thus an important determinant of genome stability (Yadav et al., PNAS, 2018).



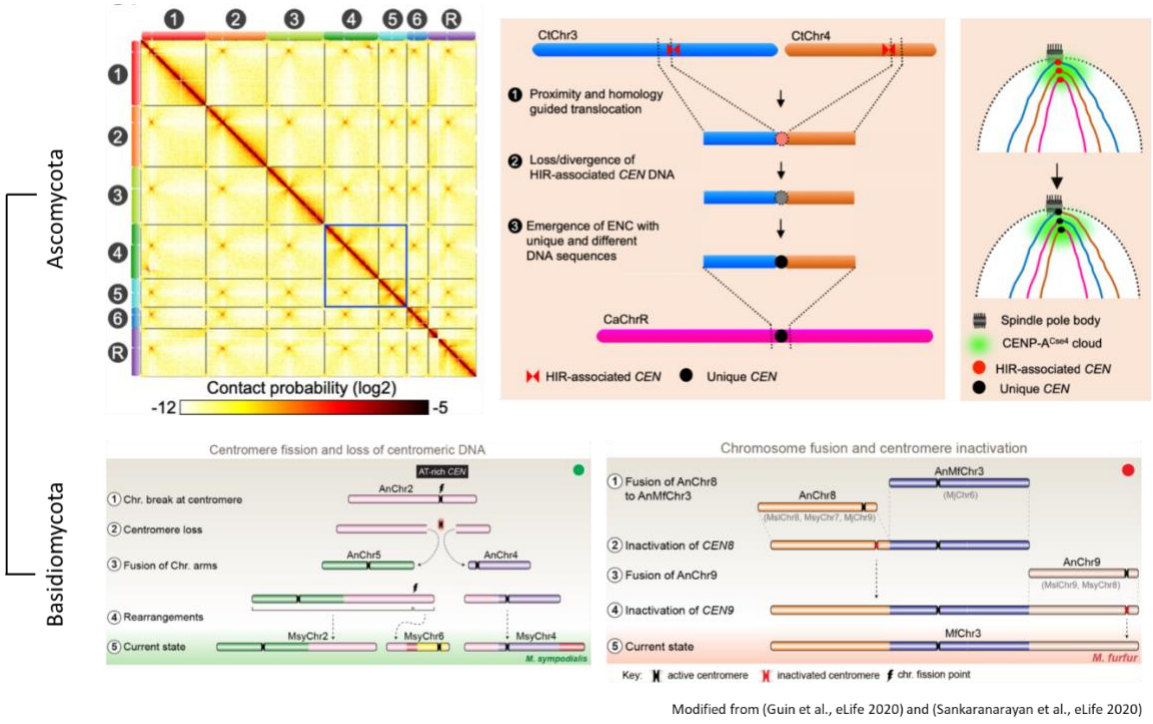


(Modified from Sreekumar et al., Genome Research 2021)

Figure 2. Epigenetic determinants of centromere assembly. (Top) Polymer model of the nucleus depicting the occurrence of early and late firing origins relative to the centromere cluster. (Bottom) A proposed role for Orc4 and Mcm2 in facilitating CENPA loading during the cell cycle.

Centromeres as the hub of trans-chromosomal contacts and hotspots of chromosomal rearrangements

The nominee's group has been involved in improving the genome assembly of a large number of medically relevant fungal pathogens including *Candida tropicalis* (Guin et al., eLife, 2020), *Candida auris* (Narayanan et al., mBio, 2021), several species of the genera *Cryptococcus* (Janbon et al., PLOS Genetics, 2014; Yadav et al., PNAS 2018), and *Malassezia* (Sankaranarayan et al., eLife, 2020). These publicly available genome/sequence data can be used to find chromosomal changes associated with drug resistance, including resistance arising due to segmental or whole-chromosome aneuploidy. Moreover, by comparing the genomes of several closely related species, the nominee's group traced the evolutionary transition events that lead to the loss or gain of a chromosome and the emergence of new species (Sankaranarayan et al., eLife, 2020; Guin et al., eLife, 2020). Centromeres are associated with inter-chromosomal rearrangements resulting in occasional centromere inactivation and reduction in chromosome number. By determining chromosomal contacts, his group established that clustered centromeres bring highly homologous centromere DNA sequences in some species into close proximity to facilitate inter-chromosomal rearrangements leading to the emergence of new species.

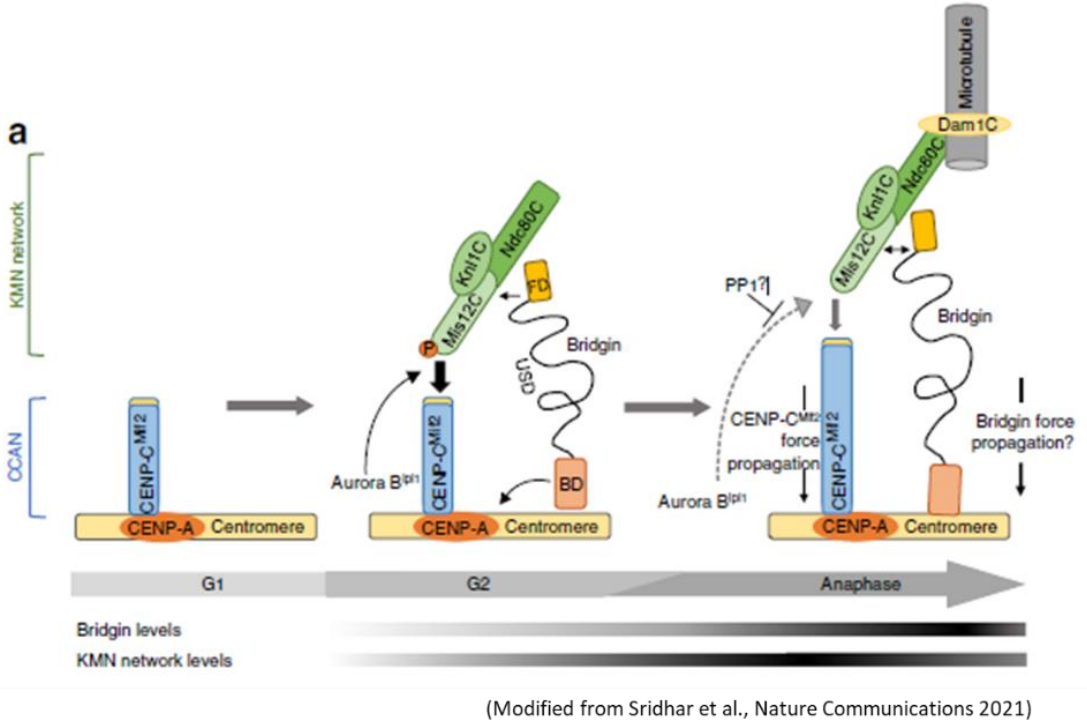


Modified from (Guin et al., eLife 2020) and (Sankaranarayan et al., eLife 2020)

Figure 3. Centromeres as a hub of inter-chromosomal rearrangements. Unique features of fungal centromeres like clustering, homology and AT-richness facilitate inter-chromosomal translocations and karyotype diversity.

Identification of novel kinetochore proteins in Cryptococcus neoformans

Cryptococcus neoformans causes life-threatening diseases including fungal meningitis. The nominee's group demonstrated that *C. neoformans* has long regional centromeres and RNAi plays an important role in the regulation of structural integrity of the centromere (Yadav et al., PNAS, 2018). By analyzing a large number of sequenced fungal genomes, many evolutionarily conserved constitutive centromere-associated network (CCAN) proteins were found to be lost in *C. neoformans* (Sridhar et al., Nature Communications, 2021). By identifying the kinetochore interactome in this organism through mass-spectrometry (MS) analyses, a set of novel kinetochore proteins including bridgin was identified. His group showed that brigdin is a unique linker protein that connects the outer to the inner kinetochore and plays a critical role in high fidelity chromosome segregation.



(Modified from Sridhar et al., Nature Communications 2021)

Figure 4. Bridgin is a novel kinetochore linker. A model describing bridgin as a kinetochore protein connecting the outer KMN network to the centromeric DNA in *C. neoformans*, in the absence of conventional linker proteins.

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