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MINI REVIEW

Chromosome stability @10!

K. T. Nishant[1] · **Kaustuv Sanyal**[2]

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Abstract
A report on the 5th International Chromosome Stability Meeting, Thiruvananthapuram, India, Dec. 14–18, 2022.

Keywords Chromosome · DNA repair · Meiosis · Mitosis · Centromere · Kinetochore

Introduction

The meeting on chromosome stability began as a series in 2012, jointly organized by the Indian Institute of Science Education and Research Thiruvananthapuram (IISER TVM) and the Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore, to facilitate interactions between the growing Indian scientific community and investigators worldwide. The 5th International Chromosome Stability Meeting was held at the IISER Thiruvananthapuram campus from 14–18 December 2022. The last decade has seen the emergence of a substantial number of principal investigators in India whose laboratories are engaged in addressing problems in chromosome stability. Around 170 scientists, graduate students, and postdoctoral fellows from Asia, Europe, the USA, and the UK working in the field of chromosome stability participated in the last meeting (Fig. 1). The meeting was spread over 4 days covering topics related to: (a) DNA replication, repair, and recombination (3R), (b) centromeres and kinetochores, and (c) genome structure and function. In addition, advances in many related areas, including the spindle checkpoint and cytoskeleton, chromosomal domains and organization, and contemporary themes like genome assembly and topology, were discussed by both early career and senior scientists. There were 45 talks scheduled in these research areas by leading investigators from India and other countries. The topics discussed in the meeting are relevant for understanding many genomic disorders, cancers, and aging resulting from chromosome instability. We present here an overview of the meeting.

DNA replication, repair, and recombination

Recent advances in DNA replication, repair, and recombination were extensively discussed in the meeting. Yathish Achar (Centre for DNA Fingerprinting and Diagnostics, Hyderabad) presented data and models depicting how the regulation of DNA supercoiling affects genome architecture and the role of Top1 and Top2 proteins in this process. Benu Brata Das (Indian Association for Cultivation of Science, Kolkata) discussed the repair of trapped Top1-DNA complexes by the Tyrosyl-DNA phosphodiesterase (TDP1). He presented data showing TDP1 methylation by arginine methyltransferase PRMT5 enhances the repair of trapped Top1-DNA complexes. The next few talks were on the theme of homologous recombination. Ganesh Nagaraju (Indian Institute of Science, Bangalore) spoke about the function of RTEL1 helicase and homologous recombination proteins at the replication fork. He showed Rad51, Rad52 levels are enhanced at the damaged forks upon RTEL1 knock down. This causes a hyper-recombination phenotype highlighting the role of anti-recombinases like RTEL1 in maintaining genome stability. Miki Shinohara (Kindai University, Osaka) spoke about the regulation of DNA end resection by Rad50 by controlling the activity of Mre11 and Sae2 nucleases. She generated several rad50 mutants and showed that some could

✉ K. T. Nishant
nishantkt@iisertvm.ac.in

✉ Kaustuv Sanyal
sanyal@jncasr.ac.in

[1] School of Biology, Indian Institute of Science Education and Research Thiruvananthapuram, Vithura, Trivandrum, Kerala 695551, India

[2] Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre For Advanced Scientific Research, Jakkur, Bangalore, Karnataka 560064, India

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Fig. 1 Group photo of the Chromosome Stability 2022 meeting

not interact with Sae2, affecting its endonuclease activity. In these mutants, non-homologous end joining (NHEJ) repair is enhanced affecting the fidelity of Double Strand Break (DSB) repair. Lucas Argueso (Colorado State University, Fort Collins) discussed the genome-wide consequences of defects in DNA replication, repair, and recombination. He showed that natural isolates of yeast strains exhibit systemic genomic instabilities leading to phenotypic diversifications over generations. These mutations show best fit to simulations of punctuated bursts of mutations rather than gradual mutations or hypermutations. Furthermore, he showed that phenotypic diversifications associated with these bursts of mutations are also stable over generations. Devyani Halder (Centre for DNA Fingerprinting and Diagnostics, Hyderabad) discussed the roles of the replication fork protection complex in response to replication stress in fission yeast. She demonstrated that DDK-dependent phosphorylation of the histone deacetylase Hst4 followed by degradation by Pop1 and Pof3 is essential for recovery from replication stress and restart of replication.

A series of talks discussed excision repair, intra-strand crosslink repair, and repair of UV damage. Umesh Varshney (Indian Institute of Science, Bangalore) discussed the uracil excision repair initiated by uracil DNA glycosylases (UDG). His work revealed that UdgX, a new UDG from *Mycobacterium smegmatis*, binds uracil very tightly and has a 4Fe-4S cluster. Additionally, the residue H109 in its signature motif acts as a nucleophile and protects the abasic site on the DNA. K. Muniyappa (Indian Institute of Science, Bangalore) discussed the role of Pso2 in interstrand crosslink repair in budding yeast. Pso2 is targeted to both the nucleus and the mitochondria, where it can repair inter-strand crosslinks. He also discussed the sequences and post-translational modifications relevant to targeting Pso2 to each of the organelles. Amit Kumar (Institute of Microbial Technology, Chandigarh) examined non-canonical phosphoinositide signaling by PIP4K2α and PIP4K2β in response to UV irradiation. These proteins exhibit nuclear localization and are frequently overexpressed in tumors. His work showed a balance in the ratio of PIP4K2α, and PIP4K2β is important. The high levels of PIP4K2β induce sensitivity to UV damage.

In a session on meiotic recombination, several talks were presented on the work performed in both yeast and mice as model systems. Viji Subramanian (Indian Institute of Science Education and Research, Tirupati) spoke about the link between double-strand break distribution and chromosome size in yeast and how the distribution of DSB density on long and short chromosomes relates to crossover density. She spoke about the localization of end-associated regions (EARs) and the higher density of breaks near chromosome ends. Homologous recombination is known to be negatively influenced by polymorphisms between parental DNA molecules. Nishant K. T. (Indian Institute of Science Education and Research, Thiruvananthapuram) presented interesting data using the SNP-ChIP analysis of the crossover protein Msh5 in meiotic cells from a yeast hybrid. He showed that the underlying polymorphisms shape the binding profile of the Msh5 protein between homologs, likely reflecting some

local heteroduplex rejection. Valérie Borde (Institut Curie, Paris) presented how DNA synthesis during the repair of meiotic double-strand breaks is not only controlled by meiosis specific factors in budding yeast but also, more generally, by DSB resection and underlying transcription. This is important since repair synthesis may be mutagenic and affect the germline. Mridula Nambiar (Indian Institute of Science Education and Research, Pune) used fission yeast to understand how cohesins prevent meiotic recombination at pericentromeric regions to avoid deleterious effects such as chromosome non-disjunction. This work is relevant to understand the causes of aneuploidy in humans, such as Down syndrome. Finally, Michael Lichten (National Institutes of Health, Bethesda) presented his new models of homologous recombination during the repair of meiotic double-strand breaks, which diverge strongly from the canonical model. For this, he analyzed by sequencing the molecular events occurring at a strong, highly polymorphic hotspot. He showed a high degree of branch migration and multiple template invasions by both DSB ends and proposed several candidate helicases that may promote these unsuspected activities.

Moving onto mouse models, Bernard de Massy (Institute of Human Genetics, Montpellier) presented work about the role of TOPOVIBL in meiotic double-strand break formation during male meiosis. He highlighted how protein–protein interactions of TOPOVIBL with SPO11 and REC114 proteins trigger meiotic double-strand break formation, using structural modeling and separation of function mutants. Subsequently, Akira Shinohara (Osaka University, Osaka) presented his work on meiotic recombination in the mouse model. He created a conditional mutant of the fidgetin-like 1 protein (FIGNL1), known to remodel or antagonize the Rad51/Dmc1 nucleofilament and prevent/limit strand invasion. In the fidgetin mutant, he found a strong accumulation of the Rad51 protein on chromosomes. These findings in meiosis also have implications for antagonizing homologous recombination in somatic cells, which is relevant for breast and ovarian cancers, often deficient in homologous recombination.

Centromeres and kinetochores

The sessions on centromeres and kinetochores covered different aspects of the structure, function, and evolution of centromeres and kinetochores in various model systems. These are essential for understanding the fidelity of chromosome segregation. Harmit Malik (Fred Hutchinson Cancer Center, Seattle) provided new insights into the evolutionary interplay between centromeres and centromere-associated proteins. Replacing the endogenous centromere-specific histone variant CID in *D. melanogaster* by either the *D. simulans* homolog, an ancestrally-inferred homolog or the *D. melanogaster* homolog as a control, the study evaluated potential mitotic or meiotic defects or defects in centromere inheritance in the mutants. The study found that homozygous F1 progeny expressing either *D. simulans* or the ancestrally-inferred versions lead to lower viability, defects in embryo development, and sterility suggesting mitotic and meiotic defects due to mal-adapted CID alleles. Geert Kops (Utrecht University, Utrecht) revealed new insights into the molecular structure of mitotic centromeres in vertebrates. He showed that mitotic centromeres in vertebrates are organized as a bi-partite structure that assembles two kinetochore microtubules. Using expansion microscopy, he described the novel finding that cohesion is found at the core centromere and stabilizes this bi-partite organization, while condensin complexes are mostly localized in the pericentromeres. Kevin Hardwick (University of Edinburgh, Edinburgh) spoke about aneuploidy in *Cryptococcus neoformans*, an important fungal pathogen, and its role in drug resistance. He showed that *Cryptococcus* cells deleted for the mitotic checkpoint kinase Bub1 show Benomyl sensitivity and exhibit slow growth. But the kinase dead strains are not very sensitive to Benomyl.

A couple of talks focussed on the kinetochores of diverse systems. Tapas Manna (Indian Institute of Science Education and Research Thiruvananthapuram) discussed morphological changes kinetochores undergo during mitosis and how this transition is regulated. He showed that ch-TOG mediated recruitment of the kinesin motor CENP-E is one of the mechanisms contributing to this transition. Dileep Verma (Northwestern University, Evanston) described that Cdt1, a pre-replication initiation complex subunit, is also a kinetochore-localized Ndc80 binding protein that synergizes with the Ska1 complex for microtubule binding. This work suggests that, like Ndc80, Ska1 is critical for recruiting Cdt1 to dynamic microtubule plus-ends, where Cdt1 facilitates a tripartite complex formation between itself, Ndc80 and Ska1. This tripartite complex could contribute towards robust coupling of kinetochores to the microtubules in mitotic cells.

Several talks were focused on the role of CENP-A, the centromere-specific histone H3 variant. Tatsuo Fukagawa (Osaka University, Osaka) provided new insights into the dynamic connectivity between kinetochore components during interphase and mitosis. He showed that in interphase, CENP-N associates with CENP-A. During mitosis, this dependency changes to CENP-C interaction with the CENP-A nucleosomes. Using Cryo-EM analyzes, the Fukagawa group characterized the complex between CENP-C and CENP-A and identified an additional CDK1-dependent phosphorylation site in CENP-C that stabilizes the complex and might be involved in the conformational switch at the kinetochore. Munira Basrai (National Institutes of Health,

Bethesda) presented a multi-organismal approach to define the causes and consequences of CENP-A/Cse4 mislocalization in yeast, human cells, and mouse models. They showed that CENP-A over-expression leads to its mislocalization to non-centromeric regions, contributing to chromosome instability. Furthermore, mislocalization of CENP-A results in aneuploidy, karyotypic heterogeneity, and increased invasiveness in cell lines and xenograft mouse models. Their studies provide the first evidence for the role of CENP-A overexpression in promoting aneuploidy with karyotypic heterogeneity and define a role for evolutionarily conserved pathways that prevent mislocalization of CENP-A in yeast and human cells. Jeyaprakash Arulanandam (University of Edinburgh, Edinburgh) addressed how the CENP-A loading machinery is targeted to centromeres. He discussed the role of the Mis18 complex (composed of Mis18α, Mis18β, and Mis18BP1), which recruits the CENP-A specific chaperone HJURP to centromeres for CENP-A deposition.

Ines Drinnenberg (Institute Curie, Paris) presented the EMBO Young Investigator Lecture on the 3D organization of holocentric chromosomes in *Bombyx mori* using Hi-C approach. She revealed that chromosomes assemble into remarkably strong chromosome territories in this organism. Zooming into the Hi-C maps of individual chromosomes, she showed A and B compartments (reminiscent of those in other eukaryotes) that organize *B. mori* chromosomes. Interestingly, the study describes a third compartment termed X that partitions about one-sixth of the *B. mori* genome and is spatially isolated within the nucleus. Finally, the OligoPaint FISH data and 3D representations of the Hi-C data reveal that X domains are more peripheral to chromosome territories than A or B domains, which might contribute to their spatial isolation.

Genome structure and function

The session's inaugural speaker was Joseph Heitman (Duke University, Durham), who summarized his group's long-standing efforts to investigate genome regulation in the pathogenic fungus *Cryptococcus neoformans*. His group found, using clinical isolates from patients, that there are hypermutator strains of the fungus in which DNA transposition is increased. Kaustuv Sanyal (Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore), whose group has been working towards understanding mitosis in fungal pathogens like *Candida albicans* for several decades, reported a new mitotic progression factor, Csa6, loss of which leads to cell cycle arrest at anaphase but over-expression of the same protein results in Mad2-mediated metaphase arrest. Continuing on the *Candida albicans* theme, the next speaker Christophe d'Enfert (Institut Pasteur, Paris) talked about his lab's extensive work on mapping genetic variations (or quantitative trait loci) that control genome copy number changes in this organism. Santanu Ghosh (Indian Institute of Technology-Bombay, Mumbai) spoke next on his group's efforts to study chromatin remodeling complexes such as RSC in the pathogenic yeast. He demonstrated that the RSC complex catalytic domain Sth1 plays a surprising role in centromere clustering in *C. albicans*. In addition, loss of this domain results in increased DNA damage, as evidenced by the accumulation of DNA damage markers such as Rad52. Karl Kuchler (Medical University, Vienna) spoke next on phenotypic switching in the pathogenic fungi *Candida auris* in skin tissues and the role of histone modifications in this process. He also showed genetic ablation of Gcn5 (a conserved histone acetyltransferase) in *C. albicans* severely affects fungal virulence in mice, indicating diminished fungal fitness in vivo. Guilhem Janbon (Institut Pasteur, Paris) spoke on alternative transcription start sites in *Cryptococcus*, showing data that suggests alternative start sites provide a robust gene regulatory potential to fungi. He showed the recent effort of his lab in screening the regulators of alternative TSS usage. Dimple Notani (National Centre for Biological Sciences, Bangalore) spoke about her group's discovery of RNA's unanticipated role in enhancing chromatin domain insulation. The findings suggest that enhancers regulate the genome organization in an RNA-dependent manner. Yamini Dalal (National Institutes of Health, Bethesda) spoke about the resetting of the epigenetic clock by histone variants in cancer and aging using powerful genomic tools and global genomic approaches. Her findings suggest the role of centromere specific histone variants (CENP-A) in regions away from the centromere where they alter the genome organization and cancer specific transcription.

In the final session on genome structure and function, Tapas Kundu (Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore) showed phosphorylation of PC4, a chromatin-associated protein, could be critically linked to genome compaction and autophagy. Shweta Tyagi (Centre for DNA Fingerprinting and Diagnostics, Hyderabad) and Shantanu Chowdhury (Institute of Genomics and Integrative Biology, Delhi) both focused on the repetitive regions of chromatin. They showed how histone methyltransferase MLL defines the fate of the human centromeres and how telomeres could regulate chromatin to activate Interleukin-1 signals over a long distance. Sabari Sankar Thirupathy (Indian Institute of Science Education and Research, Thiruvananthapuram) discussed how selection operates at the level of DNA repeat sequences to regulate gene distribution in bacteria. As EMBO global investigator, Srimonta Gayen (Indian Institute of Science, Bangalore) provided insights into how X-inactivation and reactivation happen with the transition of pluripotent states. Finally, two young investigators, Saravanan Palani (Indian Institute of Science, Bangalore) and Gunjan Mehta (Indian Institute of

Technology, Hyderabad) outlined how molecular probes and single molecular tracking can address important biological questions.

Meeting highlights and perspectives

Some of the significant new findings presented at the meeting include the mechanisms of systemic genome instability and refinements in current DNA repair and recombination models using insights from alternate model organisms and next-generation sequencing technologies. Several talks also highlighted the role of aneuploid states and other forms of genomic instability in causing phenotypic changes, drug resistance, and identifying candidate gene loci responsible for the same. Other significant insights include the loading mechanisms of centromere binding proteins, kinetochore assembly, and the molecular basis of kinetochore-microtubule interactions. In the coming years, we expect further understanding of how the 3D genome organization affects genome stability and the role of chromatin in genome organization, DNA repair, and recombination.

In addition to the talks from the invited speakers, an entire day was dedicated to a large number (85) of posters that showcased work done by graduate students and postdocs. The poster sessions were conducted at Kovalam, and both sessions were intense and interactive. Twelve poster prizes were awarded that was partially supported by the journal Chromosoma. Three recently elected members of the United States National Academy of Sciences (Michael Lichten, National Institutes of Health, Bethesda; Joseph Heitman, Duke University, Durham and Harmit Malik, Fred Hutchinson Cancer Center, Seattle) who have been associated with this conference for a long time were also felicitated at this event. Dedicated time was set aside for career opportunities by facilitating interactions between graduate students, postdocs, and potential mentors from India and other countries. Also, the DBT/Wellcome Trust India Alliance grants adviser (Devendra Singh) gave a special talk on funding opportunities for researchers in India. Towards the end, the meeting featured a panel discussion with journal editors on publishing research work.

Many international scientists who traveled to India for this meeting visited other institutes in India, presented additional talks, and interacted with graduate and postdoctoral students at these places. These visits provided further exposure to Indian students to leading investigators in the field of chromosome stability. From the organizer's point of view, it was gratifying to note the impact of this conference series since 2012 on the development of chromosome stability research in India. Interactions between participants at the earlier Chromosome Stability Meetings in 2012, 2014 (Nishant and Sanyal 2015), 2016 (Lichten et al. 2017), and 2018 have resulted in new collaborations between research laboratories, joint publications in prestigious journals, and training opportunities for many students. Some of these student participants from the earlier meetings have become independent researchers heading their own laboratories. The meeting series has, therefore, catalyzed the growth of the chromosome stability research community in India.

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Consent to participate Not applicable.

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Conflicts of interest K.T Nishant and Kaustuv Sanyal are organizers of the Chromosome Stability Meeting series. Kaustuv Sanyal is an associate editor for Chromosoma.

References

Lichten M, Nishant KT, Sanyal K, Heitman J (2017) Chromosomes in Kerala, India: 3rd Chromosome Stability Meeting (Thiruvananthapuram, December 15–18, 2016). https://biologue.plos.org/2017/04/13/chromosomes-in-kerala-india-3rd-chromosome-stability-meeting-thiruvananthapuram-december-15-18-2016/

Nishant KT, Sanyal K (2015) The good, the bad, and the ugly: how to protect chromosome stability from potential threats: a report on the Chromosome Stability Meeting, Bangalore, India, 14–17 December, 2014. BioEssays 37:717–720. https://doi.org/10.1002/bies.201500023