UNIVERSITY of GUELPH

COLLEGE of BIOLOGICAL SCIENCE

DEPARTMENT OF MOLECULAR AND CELLULAR BIOLOGY

Announcement:

All interested members of the university community are invited to attend the Final Oral Examination for the degree of ***Doctor of Philosophy*** *of*

SAFIA MAHABUB SAUTY

on Wednesday, February 28th, 2024 at 9:30a.m. (SSC 1511)

Thesis Title: Processivity Factor PCNA (*POL30*), Chromatin-Associated Factors, and Fork Protection Complex Maintain Epigenetic Stability in *Saccharomyces cerevisiae*

Examination Committee:

Dr. Angela Scott, Molecular and Cellular Biology (Exam Chair)
Dr. Krassimir Yankulov, Dept. of Molecular and Cellular Biology
Dr. Joseph Colasanti, Dept. of Molecular and Cellular Biology
Dr. Terry Van Raay, Dept. of Molecular and Cellular Biology
Dr. Patrick Lajoie, Dept. of Anatomy and Cell Biology, University of Western Ontario (External Examiner)

Advisory Committee:
Dr. Krassimir Yankulov (Adv)
Dr. George van der Merwe
Dr. Joseph Colasanti
Dr. Rebecca Shapiro

Abstract: The transcriptional profile of a eukaryotic cell is influenced by its genome organization and chromatin landscape. Chromatin structures and their associated epigenetic marks are faithfully inherited in subsequent cell divisions to maintain cellular identity. Dysregulation or disruptions in this process are associated with several human diseases, however, the underlying mechanisms are poorly understood. In this thesis, I investigated the role of the DNA processivity factor PCNA (*POL30*) in maintaining transcriptional silencing using *S. cerevisiae* as a model organism. Using combinations of low and high-sensitivity loss-of-silencing assays at the *VIIL* sub-telomere, I showed that mutations in *POL30* (*pol30-6*, *pol30-8*, and *pol30-79*), along with the deletions of histone chaperone *CAC1* and helicase *RRM3* cause transient de-repression. The deletion of chaperone *ASF1* caused epigenetic conversion, meaning a persistent shift from the silent to the active state of the gene. Biochemical assays showed differential physical interactions of the mutant pcna proteins with Cac1p and Rrm3p. Analysis of the *FLO* loci responsible for flocculation in yeasts showed that the deletions of fork stabilizing factors *TOF1* and *RRM3* reconstitute mild flocculation in *pol30* mutant strains. The increase in flocculation correlated with increased *FLO11* expression and epigenetic conversions at this locus. Parallel investigation of the SIR-dependent loci demonstrated similar effect of these factors at the sub-telomeres and the mating-type loci. Data from this thesis strongly indicate that mutations in PCNA (*POL30*) predispose to heritable epigenetic instability that can result in epigenetic conversion upon disruptions in the nucleosome assembly pathway. Additionally, the effect of PCNA-mediated interactions of *TOF1* and *RRM3* indicates a mechanism-

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independent regulatory role of these factors. Finally, this thesis challenges the interpretation of the loss-of-silencing assays traditionally used in the field of yeast epigenetics.

Curriculum Vitae: Safia obtained her Bachelor of Science (Honors) in Microbiology at the University of Dhaka, Bangladesh in 2019. In the spring of 2019, she entered into the MSc. program at the University of Guelph under the supervision of Dr. Yankulov. In the summer of 2020, Safia transferred to the PhD program.

Awards: CBS International PhD Graduate Research Assistantship 2020-2024
International Doctoral Tuition Scholarship 2020-2024

Publications: **Sauty, SM.**[#] and Yankulov, K. Mutations in the DNA processivity factor PCNA (*POL30*) predispose to epigenetic instability at the *FLO11* locus in *S. cerevisiae*. In peer review.

Sauty, SM. and Yankulov, K. (2023). Analyses of *POL30* (PCNA) reveal positional effects in transient repression or bi-modal active/silent state at the sub-telomeres of *S. cerevisiae*. Epigenetics and Chromatin. 16 (1), 40.

Shaban, K., Dolson, A., Fisher, A., Lessard, E., **Sauty, SM**., Yankulov, K. (2023). *TOF1* and *RRM3* reveal a link between gene silencing and the pausing of replication forks. Current Genetics, 1-15.

Shaban, K., **Sauty, SM.**, Fisher, A., Cheng, A., Yankulov, K. (2023). Evaluation of drug-free methods for the detection of gene silencing in *S. cerevisiae*. Biochemistry and Cell Biology 101 (1), 125-130.

Dolson, A., **Sauty, SM.**, Shaban, K., Yankulov, K. (2021). Dbf4-Dependent Kinase: DDK-ated to post-initiation events in DNA replication. Cell Cycle 20 (22), 2348-2360.

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Sauty, SM.*, Shaban, K.*, Yankulov, K. (2020). Gene repression in *S. cerevisiae*—looking beyond Sir-dependent gene silencing. Current Genetics 67 (1), 3-17.

#corresponding author *co-first author