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Master Thesis Defense

Entitled

INVESTIGATING THE ROLE OF IRC20 AND CDC48 IN THE MAINTENANCE OF YEAST GENOME

by

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Date & Venue

1:00 pm

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Fatima Theatre

2C021 located on 2nd floor 'C' block female side.

Abstract

DNA, the basic unit of inheritance, is continuously exposed to numerous endogenous and exogenous damaging agents that compromise its function and adversely impact health. However, the cell has evolved intricate DNA repair mechanisms to mitigate the negative consequences of genomic instability. The repair of DNA double-strand break (DSB) is potentially accomplished by various mechanisms, including homologous recombination (HR). An HR-mediated DSB repair requires a controlled regulation of repair protein to ensure timely function. Posttranslational modifications (PTMs), such as ubiquitylation and SUMOylation, are regulatory mechanisms that significantly fine-tune proteins' activities. Central to these processes is Rad52, a conserved oligomeric protein that forms a ring-shaped structure and primarily serves as a target for SUMOylation, stimulated by DNA damage. In Saccharomyces cerevisiae, Irc20 is a SUMO-targeted ubiquitin ligase that plays a pivotal role in controlling Rad52 enrichment at DSB. Interestingly, the multifunctional mediator of the ubiquitin-proteasome system (UPS), Cdc48 protein, is also important for modulating the levels of Rad52. However, the interaction between these proteins along with the crosstalk of PTMs pathways remains enigmatic. This thesis aims to investigate the mechanism of action of Irc20 and Cdc48 in regulating Rad52 protein. In addition, we examine the functional significance of their interaction in the maintenance of the genome during DNA repair and beyond. In this study, using purified Irc20 and Rad52, we show that Rad52 is a potential substrate of Irc20 in vivo, and Rad52 SUMOylation triggers its subsequent ubiquitination. In addition, we report a genetic interaction between IRC20 and CDC48 suggesting that Irc20 is functioning upstream of Cdc48. Furthermore, our work identifies a role of Cdc48 in recycling Rad52 from DSB sites during recombination. Importantly, we uncover a novel role of Cdc48 in the maintenance of the 2-µm plasmid copy number. Altogether, our findings support a model in which Irc20 facilitates Cdc48 in Rad52 regulation.

Keywords: *Saccharomyces cerevisiae*, DNA repair, Irc20, Cdc48, Rad52, ATPase, ubiquitin ligase, 2-µm plasmid, recombination, ubiquitin, SUMO.