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ROLE OF THE SNF2 HOMOLOG, IRC20, IN YEAST GENOME MAINTENANCE

by

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Abstract

In eukaryotes, DNA is wrapped around histone proteins forming a highly compact structure, the chromatin. All DNA-based processes must occur within the complex organization of the chromatin, and this requires modulation of its structure when needed. This is accomplished by covalent histone modifications that alter histone-DNA contacts, as well as through the actions of ATP-dependent chromatin remodelers. These multi-subunit complexes play major roles in transcription regulation, replication and repairing DNA damage. This thesis aims to characterize a poorly studied member of the SWI/SNF family of ATPases/helicases, Irc20, from *Saccharomyces cerevisiae*. Previously, Irc20 has been shown to be involved in recombinational repair and to possess ubiquitin ligase (E3) activity. The human homolog of Irc20, SHPRH, has also been implicated in repair via the poly-ubiquitylation of PCNA, the sliding clamp of the DNA polymerase. Loss of heterozygosity in the region containing the SHPRH gene is seen in a wide variety of cancers. In this study, using purified Irc20, we showed that it possesses DNA and nucleosome binding activities, as well as an ATP-hydrolyzing activity. However, despite homology to Snf2 catalytic domain, Irc20 did not have the ability to alter chromatin structure. Using point mutations in different Irc20 domains, we identified that the increased recombination centers observed in *irc20* null mutants is dependent on both its ATPase and ubiquitin ligase activities. Consistent with this, we observed higher recruitment or retention of the recombination repair factor Rad52 at a single induced double strand break in *Δirc20* mutant, suggesting a regulatory role for Irc20 in DNA repair. Furthermore, we observed a previously unidentified function for Irc20 in regulating the levels of the endogenous yeast 2-µm plasmid. In *irc20* null mutant, we observed a three to four-fold increase in 2-µm levels, forming high molecular weight forms in a manner dependent on homologous recombination. We suggest this is, at least partially, through regulating the levels of Flp1 recombinase since we observed higher levels of Flp1 in *Δirc20* mutant after shutting off expression from a repressible promoter. Collectively, our results show a regulatory role for Irc20 in recombination underlying its role stabilizing the genome and regulating the 2-µm plasmid levels.

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