Jakub Muraszko

“Biochemical properties of Rrp1important for genome stability maintenance in *Schizosaccharomyces pombe*.”

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Promotor: dr hab. Dorota Dziadkowiec

Abstract

The process of homologous recombination is a complex and extremely important mechanism ensuring genome stability. Research on its course allows us to understand replication, exchange of genetic information, and DNA repair. One of the factors involved in the process of homologous recombination in the model organism *Schizosaccharomyces pombe* yeast is the complex of proteins Rrp1 and Rrp2. Previous studies have confirmed its significant influence on the maintenance of the genetic stability of *S. pombe* cells.

In this study, a detailed analysis of the interaction of Rrp1 with Rad51 recombinase, which is the main protein involved in homologous recombination, was performed. The first part of the work shows the ability of Rrp1 protein to regulate Rad51 in *S. pombe* cells. Rrp1 rescues the toxic overproduction effect of Rad51 and affects its localization. Rrp1 is located in the same regions as Rad51 and forms a complex with it. The Rrp1 translocase and, to a lesser extent, its ubiquitin ligase activity turned out to be important for these processes. Moreover, the interaction of Rrp1 with the RPA complex suggests the regulation of Rad51 within the replication forks.

In the second part of this work, a number of biochemical properties of Rrp1 *in vitro* were examined. The purification procedure for recombinant Rrp1 protein in a bacterial system was successfully optimized. Rrp1 has been shown to be capable of DNA-dependent ATP hydrolysis, as well as binding to single- and double- stranded DNA. The direct interaction of Rrp1 with Rad51 is shown by *in vitro* immunoprecipitation, and specific region of this interaction was recognized. The influence of Rrp1 on the formation of Rad51-DNA complexes was analysed and the ability of Rrp1 to remove recombinase from the double stranded DNA was demonstrated. Rrp1 influences the Rad51 driven strand exchange reaction, which was reconstructed *in vitro*. Rrp1 possesses ubiquitin ligase activity and is able to ubiquitinate Rad51 in an *in vitro* process, dependent on Uba1 and Ubc4.

Data collected from both *in vivo* and *in vitro* systems allowed me to propose a model for the interaction of Rrp1 with the Rad51 recombinase.