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The role of DNA dependent translocases in maintaining genome stability in *Schizosaccharomyces pombe*

ABSTRACT

Helicases are a group of proteins that move along nucleic acids. Their basic function is to unwind double-stranded DNA, so that single strands can be separated. This group of proteins includes translocases, which use their ATPase activity to move along DNA strands, but not to unwind them. Helicases participate in DNA replication and repair processes. The Sf1a helicase family includes the DNA-dependent Srs2 helicase in *S. cerevisiae* and *S. pombe*. The homologous recombination proteins in *S. pombe*, Rrp1 and Rrp2, belong to the a Rad5/16-like group of Snf2 translocases. Rrp1 and Rrp2 act in the pathway dependent on homologous recombination mediators, Swi5/Sfr1, together with Srs2 in response to stress associated with DNA repair and/or replication. In this study, the role of the Srs2, Rrp1 and Rrp2 proteins in response to replication stress was compared.

In the first part of the work, it was shown that increasing the amount of Srs2, similarly to Rrp1 and Rrp2, leads to inhibition of cell growth, chromosomal instability and replication stress. It was shown that overproduction of Srs2, Rrp1 and Rrp2 activates checkpoints in different ways. Previous studies uncovered the involvement of Rrp1 in the regulation of Rad51 recombinase. In this work, it was shown that excess Srs2 exacerbates the negative effects of Rad51 overproduction, in contrast to what was observed for Rrp1. This suggests that Srs2 in *S. pombe* regulates Rad51 differently than Srs2 in *S. cerevisiae.*

In the second part of the work, the differences in functions of the studied proteins in postreplication DNA repair in *S.pombe* were demonstrated. Depending on the stress factors used, it was shown that Rrp1 and Rrp2 inhibit the repair pathway dependent on homologous recombination in postreplication DNA repair, but without the participation of Srs2, this pathway is not fully functional. Rrp1 and Rrp2 can also act on the pathway associated with template switching, like Rad8. On the other hand, Srs2 in *S.pombe* does not inhibit the repair pathway dependent on homologous recombination which is the role of its *S.cerevisiae* ortologue.

Srs2 in *S.pombe* lacks the C-teminal fragment containing PIM and SIM motifs in *S.cerevisiae* ortologue. Rrp1 has both of these motifs. In the third part of this work, *in vivo* and *in vitro* studies showed that Rrp1 interacts specifically with PCNA (excluding the binding of the studied proteins via DNA), and Rrp1 can also act as an E3 ubiquitin ligase, whose substrate is PCNA. It is possible that Rrp1 participates in the removal of PCNA from DNA.

Obtained results increased our knowledge on the role of studied proteins in maintaining genome stability in *S.pombe*.