

STRUCTURE-FUNCTION ANALYSIS OF YEAST RAD18

Orsolya Frittmann, Vamsi Krishna Gali, Ildikó Unk

Biological Research Centre of Szeged, Institute of Genetics, 6726 Szeged, Temesvári krt. 62., unk.ildiko@brc.mta.hu

1. INTRODUCTION

Damage to DNA can stall replication forks which can lead to cell death. In yeast the DNA damage tolerance (DDT) pathways help bypass DNA lesions so that replication can proceed forward.

In the yeast *Saccharomyces cerevisiae* DDT is controlled by the Rad6-Rad18 complex. It can be divided into three different subpathways, based on genetic experiments.

(I). The error prone translesion polymerase subpathway which includes Def1, Pol zeta, Rev1.

(II). The error free translesion polymerase subpathway which includes Pol eta.

(III). The error free fork regression subpathway which includes Rad5, Mms2, Ubc13.

During replication the replicative DNA polymerase is bound to proliferating cell nuclear antigen (PCNA) that acts as a processivity factor. Upon stalling of the replication fork at a DNA damage, the Rad6-Rad18 ubiquitin conjugase-ligase complex ubiquitinates PCNA. Monoubiquitination of PCNA activates the error prone and error free translesion polymerase subpathways, while polyubiquitination activates the fork regression subpathway.

Since Rad18 plays an essential role in the DNA damage tolerance pathways, to better understand its function we carried out a structure function analysis of Rad18. The Rad18 protein has five identified domains and the function of only three has been established so far. These are the Really Interesting New Gene (RING), ATP- and Rad6 binding domains. The functions of Zn-finger and SAP domains have not been investigated. There are also regions in Rad18 which remain uncharacterized.

2. MATERIALS AND METHODS

Gene replacement for making deletion strains.

Yeast two hybrid to study protein interactions.

Measurement of spontaneous and induced mutagenesis.

Sensitivity assays.

3. RESULTS

3.1. Constructed Rad18 deletion mutants

We constructed a series of deletion mutants, three of them have deletions in the C-terminal region, three in the N-terminal region, all of which comprise previously uncharacterized regions of Rad18. The other four deletion mutants comprised the Zn-finger and SAP domain, together or separately, whose function has not been investigated so far in yeast Rad18.

3.2. DNA damage sensitivity assays to analyse the deletion mutants

We introduced the plasmids carrying the Rad18 deletion mutants on expression vectors to a strain in which we deleted the genomic copy of *RAD18*. We checked the DNA damage sensitivity of these strains to examine how the deletions affected the function of Rad18. In these assays we applied ultraviolet light (UV) which induces thymine dimers, methyl-methane sulfonate (MMS) which methylates the cytosine, and hydroxyurea (HU) which depletes the dNTP level and causes replication defects.

The three C-terminal deletion mutants did not show any sensitivity to the above mentioned DNA damaging agents when compared to the wild type, which indicates that they have no role to play in the function of Rad18. However, one of the N-terminal deletion mutants, Rad18(80-190) showed sensitivity similar to a complete Rad18 deletion, while the other two showed intermediate sensitivity when compared to the wild type. Deletion of the Zn-finger domain or the Zn-finger and SAP domain combined conferred sensitivity similar to that of the complete Rad18 deletion.

3.3. Epistasis analysis of Rad18(115-190)

We investigated further the N-terminal deletion mutant Rad18(115-190) which showed intermediate sensitivity upon DNA damage. We performed an epistasis analysis to determine which one of the three the DNA damage tolerance subpathways was affected by the deletion. Double deletions of the Rad18(115-190) mutant and

members of the three subpathways of DDT were examined. Deletion of Rev1 in Rad18(115-190) mutant showed a non epistatic relationship which indicated that the mutant functioned outside this subpathway. Similarly, a non epistatic relationship was observed with Rad30. But deletion of Mms2 in the Rad18(115-190) mutant did not confer an additional sensitivity, which indicated that the mutant might function in the Mms2-Rad5 subpathway.

3.4. Spontaneous and UV induced mutagenesis of Rad18(115-190)

To further confirm that the mutant played a role in the Mms2-Rad5 subpathway we checked spontaneous mutagenesis in the mutant. Our results showed an elevated level of spontaneous mutagenesis in Rad18(115-190) when compared to wild type, which is characteristic of the inactivation of the Mms2 subpathway and is in good agreement with the epistasis analysis.

3.5. Yeast two hybrid experiment with Rad18(115-190)

Our results indicated that the Rad18(115-190) mutant played a role in the Rad5-Mms2 subpathway of DDT. Rad18 was already known to physically interact with Rad5. Therefore we examined the possibility that the deletion disrupted the interaction, in yeast two hybrid analysis. The results showed that the mutant Rad18 was not capable of interacting with Rad5. This indicates that the region deleted in Rad18(115-190) mutant mediates the interaction with Rad5.

4. CONCLUSION, SUGGESTIONS

We have successfully constructed and examined the sensitivities of eight deletion mutants of Rad18. We established that the Rad18(115-190) mutant affects the interaction of Rad18 with Rad5. Our studies also indicate that the region deleted in Rad18(80-190) affects the interaction with PCNA. We obtained results that showed that deletion of the Zn-finger completely abolished the function of Rad18 indicating an essential function of this region.

5. REFERENCES

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