The role of translesion synthesis DNA polymerases during transcription (K 109521)

Our project focused on the investigation of yeast Pol eta. By uncovering a transcription elongation function of Pol eta, and by identifying a specific RNA synthetic activity of the enzyme, our results can significantly change the conception about the mostly unknown territory of RNA translesion synthesis, and show a new direction of research of TLS DNA polymerases.

The results of the project are listed according to the research plan.

I. Confirmation of the involvement of Pol eta in transcription.

1. Genetic analysis of double deletion mutants of *RAD30*. By engineering mutant yeast strains we examined the genetic relations of *RAD30* to genes functioning in transcription (*DST1, SPT4, RPB4, RPB9, ELP1, RAD26, SNF2, SNF5, SNF6, DEF1*). Applying transcription inhibitors (6-azauracil, mycophenolic acid), we could show epistasis between *RAD30* and elongation factor genes, whereas a non-epistatic relationship was observed with genes functioning in other steps of transcription. These results suggested that Pol eta might work during transcription elongation.

2. Direct measurement of RNA synthesis in *rad30* strains. The involvement of Pol eta in transcription was confirmed by measuring mRNA levels transcribed from constitutive (*UBC6, TRP3, TAF10*) and inducible (*IMD2, GAL1, GAL10*) promoters in RT-qPCR experiments. We showed that transcription levels of these genes decreased in the absence of *RAD30*. The results obtained with the mutant strains reflected the genetic relationships observed in the above mentioned genetic analysis. Enzyme activity measurements in the mutant strains using the dual-luciferase reporter system yielded similar results. Interestingly, using a strain expressing the enzymatically inactive D30A Pol eta in these assays yielded similar results as the *rad30* deletion strain.

II. Analysis of the role of Pol eta in specific steps of transcription.

1. Examination of association of Rad30 with specific regions of a gene. In chromatin immunoprecipitation (ChIP) experiments Pol eta, labeled with 9-myc tag at the C-terminus, showed preferential enrichment over the ORF of the *GAL1* gene after galactose induction. Contrary, no enrichment was observed without induction, or over two independent intergenic regions. Control ChIP experiments with Gcn5-9Myc, Snf5-9Myc, and with PolII strengthened these results. ChIP data with a strain having the catalytically inactive D30A Pol eta revealed a decrease in the association of PolII with the 5' region of the ORF of *GAL1*. Additionally, direct analysis of transcription elongation on chromatin in G-less based run-on assays (GLRO) showed a substantial decrease in elongation efficiency in the lack of Pol eta. These data indicated the involvement of Pol eta in elongation.

2. Identification of interacting partners of Rad30. A. Tandem affinity protein (TAP) purification was performed with a strain expressing C-terminally TAP-tagged Pol eta. Despite repeated efforts, only Pol eta could be recovered in the final elution step of the purification. Considering that the C-terminal TAP tag could interfere with interactions, we repeated the experiment with another strain having N-terminally TAP-tagged Pol eta. With this strain we could detect a few faint bands beside Pol eta in the final elution. However, the low signal indicates that the different steps of

purification need to be further optimized. **B.** Yeast two hybrid library screens identified several interaction partners of Pol eta, and the specificities of these were confirmed in direct two hybrid assays. However, we have not been able to establish an obvious connection between the identified genes and Pol eta. Further examination of these genes is in progress.
In a more direct approach to show interactions of Pol eta with transcription factors, we chromosomally tagged elongation factor genes with affinity tags in the strain expressing 9Myc-tagged Pol eta. Co-immunoprecipitation experiments using these strains are ongoing in our laboratory.

III. Investigation of the involvement of Pol eta in the transcription of UV-damaged DNA.
1. *In vivo* transcriptional fidelity. These experiments are still in progress using semi *in vivo* conditions by applying transcription competent cell extracts and plasmids carrying modified bases at defined locations.
2. *In vitro* RNA synthesis by Rad30. We applied *in vitro* oligonucleotide extension assays with purified Pol eta to investigate, whether Pol eta could carry out RNA synthesis. These assays proved that Pol eta could extend RNA primers with ribonucleotides. Steady state kinetic measurements indicated that Pol eta recognized RNA as its substrate preferentially inserting ribonucleotides into RNA compared with DNA. Moreover, Pol eta could synthatize RNA opposite 8oxoG, the most frequent spontaneous oxidative DNA lesion, and TT dimer, the most frequent UV-induced DNA lesion. Moreover, it did it in error-free way inserting the correct C opposite 8oxoG, and A opposite the TT dimer. However, kinetic analysis revealed that under conditions mimicking the intracellular nucleotide concentrations, Pol eta inserts rNTPs and dNTPs with similar efficiencies during RNA extension opposite undamaged templates, and it inserts dNTPs ~20 times more efficiently than rNTPs opposite damaged nucleotides.

IV. Examination of the possible involvement of other TLS polymerases in transcription.
Investigation of the genes of the other TLS polymerases, *REV1*, *REV7*, and *REV3* could not identify a strong phenotype upon treatment with transcription inhibitors. Therefore, we investigated other non-polymerase members of the Rad6/Rad18 pathway. First we examined PCNA an essential interaction partner of Pol eta during DNA synthesis to rule out the involvement of the replicative function of Pol eta in transcription. Mutational analysis of PCNA did not result in increased MPA sensitivity in the mutants. Nevertheless, characterization of mutants exhibiting increased DNA damage sensitivity led to the identification of a new regulatory surface at the inter-domain boundary of PCNA regulating several DNA repair pathways.
We investigated the *RAD18* gene, as its deletion resulted in increased MPA sensitivity. To identify which activity of Rad18 was responsible for the sensitivity, we generated a series of deletions removing different parts or domains of the protein. Our results were presented at an international conference last year.
We also examined other proteins as detailed in the report after the 3rd year. These investigations are still ongoing.