The scope of my project was to understand how the DNA double-strand damages affect the regulation of RNA polymerase II (RNAPII) transcription on distinct subsets of target genes (inhibited and transcribed). Between March and July 2018, I was working in the IGBMC in the lab of Evi Soutoglou and Laszlo Tora. I had a chance to join into their common project with Haico Van Atticum from Netherland, in which we investigated the RNAPII behavior during and after different DNA damage inducing conditions, by using chromatin immunoprecipitations. We identified a new E3 ubiquitin ligase, WWP2, which participates in RNAPII degradation. Additionally, we also induced DNA double-strand breaks and analyzed the RNAPII behavior by live cell imaging. During the period I was spending in Strasbourg I was succeeded in two different ways:

1. I could join to a project in which we showed that WWP2 was necessary for the DNA damage induced transcriptional silencing by mediating RNAPII ubiquitylation and its proteasomal degradation at the damaged site. From these results, we have already prepared a manuscript form the data, which will be planned to submit in the Nature Cell Biology in August.

2. I had a chance to join to a laboratory, where the principal investigators are ERC grant winners (Evi Soutoglou – ERC consolidator grant, Laszlo Tora – ERC advanced grant). They supported me to plan a new project which will be submitted as an ERC-COG grant at the beginning of 2019. Additionally, I had a chance to establish a new international collaboration with Haico Van Atticum (LUMC-Netherland) who is also an ERC-COG grant winner.

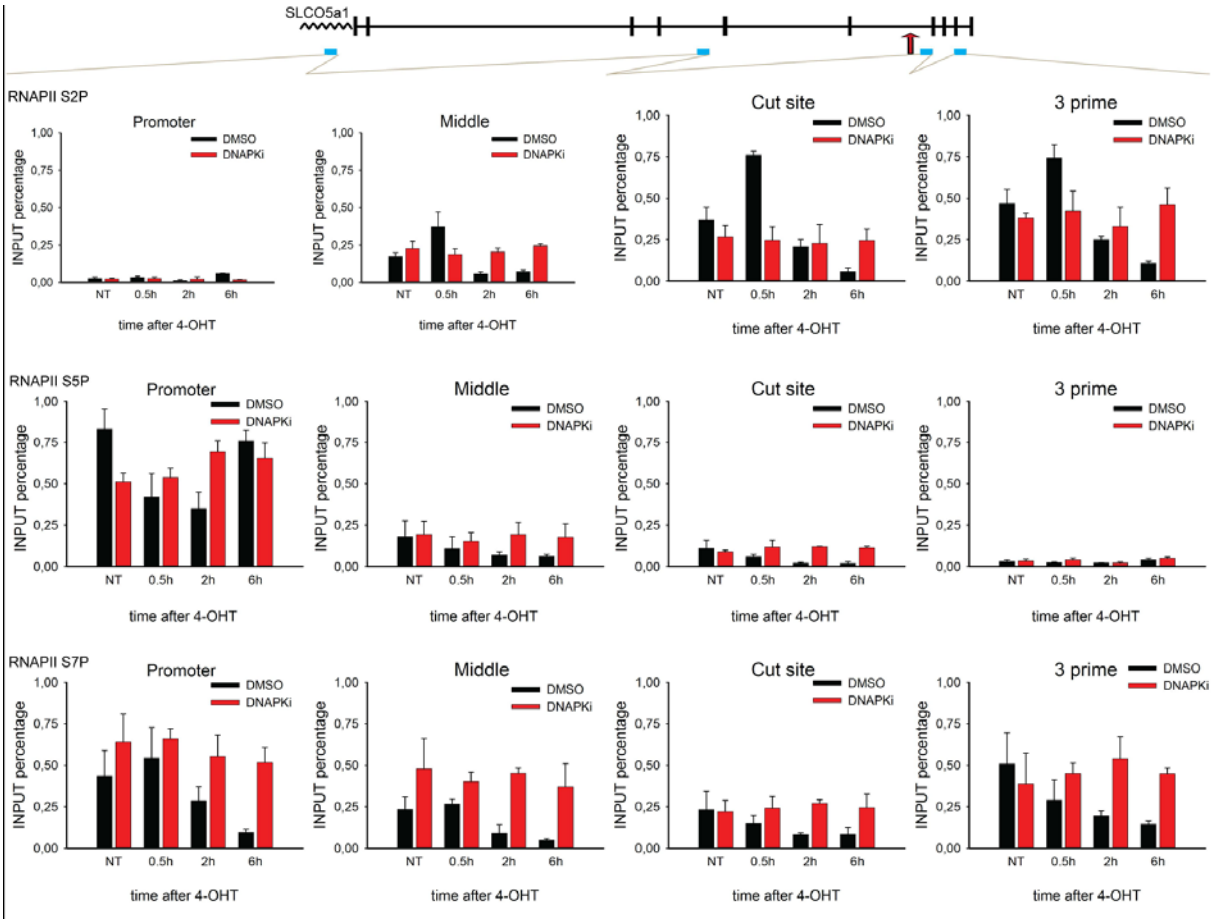
During the implementation period I also had some difficulties: However, the project started in March 2018, I got the contract only at the end of May. Finally, the reference number of my project was evaluated at the beginning of June (the last month of the project). However, I could finish the project in spite of the following financial problems occurred:

1. I could not pay my accommodation from the budget, since the it was not transferred in time to the account of the University of Szeged. I needed to rent a house from my daily-fee budget.

2. Instead of 4 months (16 weeks) I could stay only 10 weeks with breaks in Strasbourg since I could not estimate when the project funding would be started (therefore, I had to pay it from my own budget).

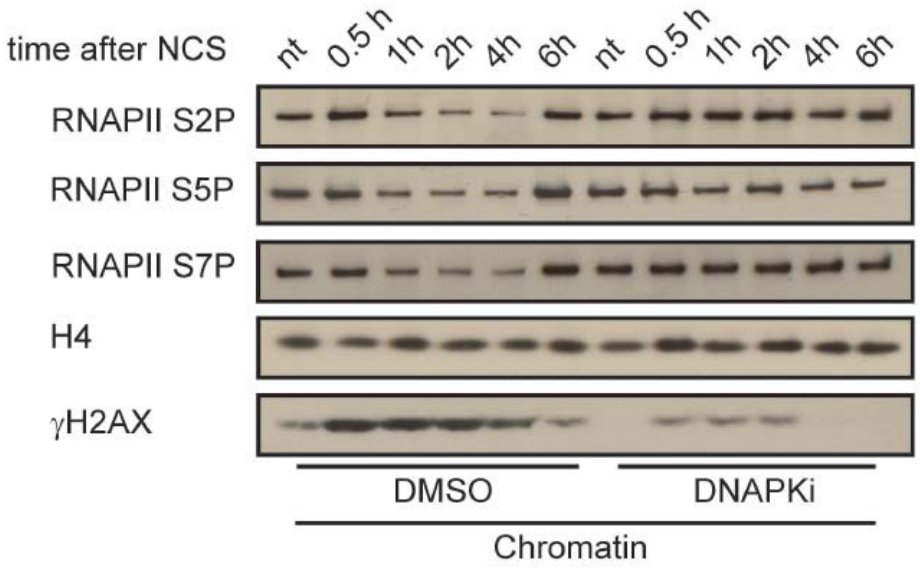
The following new data could be obtained from the project:

We monitored the initiating and elongating RNAPII distribution a short time before and several hours after the break induction at the coding region of *Dab1* and *Slco5a1*. We checked the RNAPII distribution by monitoring the S2, S5 and S7 phosphorylation of the RPB1 CTD (the C-terminal domain of the largest subunit od RNAPII) by performing chromatin immunoprecipitation experiments. In accordance with the published results before the break induction we could detect elevated level of S5P at the promoter region of *Slco5a1* and *Dab1*, increased occupancy of S2P at the gene body, while the S7P showed uniform distribution at the promoter and the gene body, as well. Short time after the DSB induction the binding of RNAPII is decreased at the *Slco5a1* and *Dab1* genes. The process affects both the transcription initiation and elongation, since reduced RNAPII binding can be observed at both the promoter and gene body. By using inhibitors against DNAPK we could prevent the RNAPII dissociation from the damaged gene. We detected that RNAPII CTD S5P and S7P followed the same kinetics during DSB induction, since the binding of both phosphorylated forms of RNAPII are dramatically reduced at the examined gene regions. On the contrary we could detect an increase in the distribution of S2P RNAPII short-time after the break induction, which was decreased only 3h after DNA damage induction. The inhibition of DNAPK resulted in a loss of S2P RNAPII protein level, which suggests that DNAPK kinase activity could affect the phosphorylation state of the elongating RNAPII. However, the RNAPII dissociation or the S2P hyper-phosphorylation is restricted to only the damaged gene, since identical RNAPII distribution could be observed in case of the surrounding genes show under all experimental conditions. Based on these results it seems that when DSB occurs at a transcribed region the DNAPK-mediated hyperphosphorylation of RNAPII appears as an early step in transcriptional silencing. In addition, the distribution of unphosphorylated RNAPII was also investigated by performing chromatin immunoprecipitation.



Since the proteasomal degradation is linked to K48 chain specific ubiquitylation in our next step we tried to identify the ubiquitin ligase, which is responsible for RNAPII ubiquitylation under the above described conditions. To distinguish whether the RNAPII dissociates from the damaged genes or it is degraded at the break site, we fractionated cell lysates, in which we separated the cytoplasmic, the nuclear soluble and the chromatin fractions of the cells. We monitored the RNAPII distribution before and after DSB induction by using the radiomimetic drug neocarzinostatin. In the chromatin bound fraction we could detect the same kinetics as observed in the ChIP experiments: the binding pattern of S5P and S7P RNAPII showed decreased kinetic upon DSB induction, while the S2P RNAPII level was increased 30 min after the break induction, then it was decreased 1 h later. By using DNAPK inhibitor the S2P, S5P and S7P RNAPII remained in a constant level during the entire experiment. As a following step we checked whether RNAPII was dissociated form the damaged gene and it was transported into the nuclear soluble fraction. However, we could find any increase in the level of neither the unphosphorylated nor the phosphorylated forms of RNAPII in the nuclear soluble fraction, it was decreased in the chromatin bound fraction upon DSB induction. Finally, using short interfering RNA (siRNA)-mediated silencing of DNAPKcs supported the effects observed by

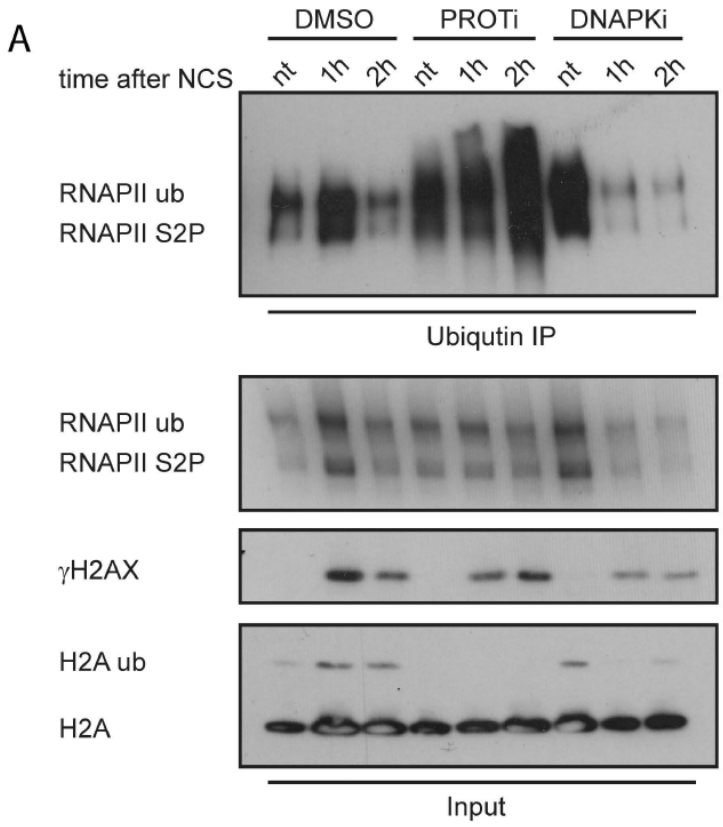
DNAPK inhibition at the damaged genes. As expected the reduction in the level of DNAPKcs rescued the RNAPII level after DSB induction. These and our previously published data suggest that RNAPII is somehow targeted for proteasomal degradation at the site of the damage.



According to these data it seems that the RNAPII is probably ubiquitylated to halt the transcription process during DSBR, thereby avoiding the transcription-DNA repair collision. To prove that the RNAPII is ubiquitylated we performed an immunoprecipitation experiment, in which we immunoprecipitated the ubiquitin-bound proteins by using TUBE resin and we checked whether the RNAPII was present in the ubiquitinated protein pool. For western blot detection of RNAPII first we used an antibody which recognizes all form of RNAPII. Surprisingly, when we used an antibody for detecting the S2P RNAPII we could observe elevated level of this form upon DSB induction, which suggests that the S2 phosphorylated RNAPII is a better substrate for ubiquitylation-driven degradation. Our previous data suggested that the RNAPII degradation happened short-time after the break induction, therefore we tested the ubiquitylation pattern under normal conditions and 1 or 2 hours after DSB induction. We could detect a significanincrease in DMSO-treated (used as a vehicle control) samples, but it was elevated 1 hour after the break induction. This suggests that RNAPII was ubiquitylated immediately after the break repair had taken place. On the other hand, 2 hours after the break induction, RNAPII ubiquitylation level was slightly decreased suggesting that RNAPII was degraded by the proteasome.

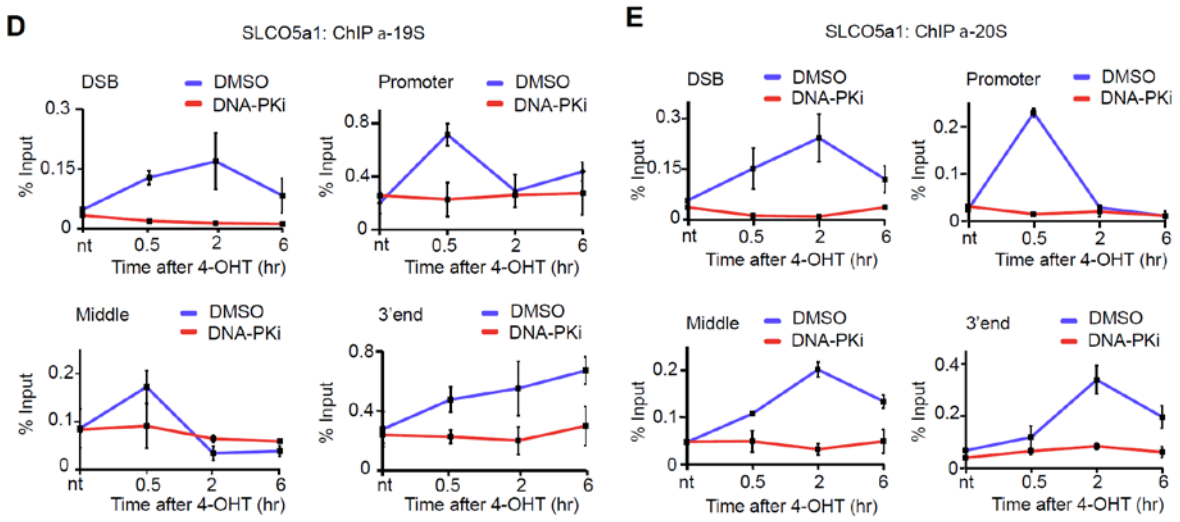
To prove that the disappearance of ub-RNAPII is mediated by the proteasome we treated the cells with proteasome inhibitor. As we expected the proteasome inhibitor prevented the degradation of the ubiquitinated RNAPII which clarified that the reduction in the level of ub-

RNAPII was the consequence of its ubiquitin-mediated proteasomal degradation. Finally, by using DNAPK inhibitor we couldn't detect higher level of ub-RNAPII during DSB damage compared to the level observed under normal condition suggesting that the DNAPK is necessary for the regulation of RNAPII ubiquitylation.

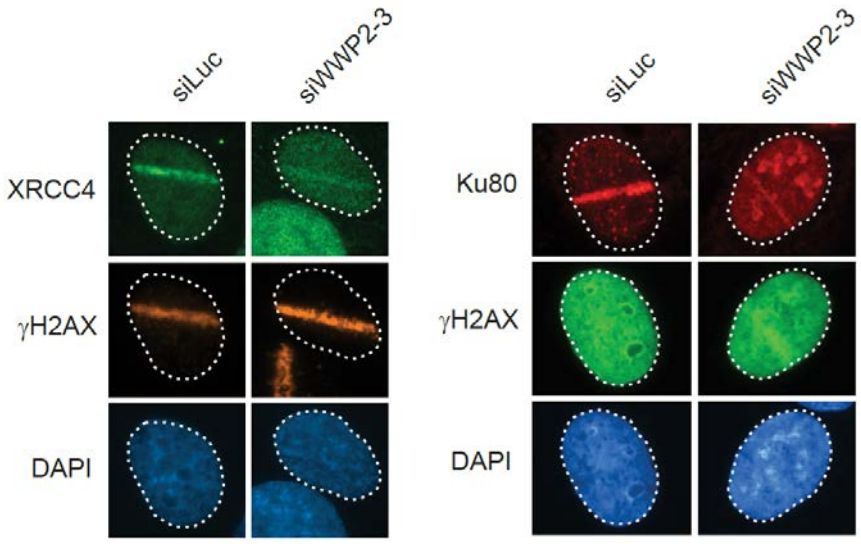


To support the results observed in case of DNAPK inhibition and to further test whether the E3 ubiquitin ligases were indeed involved in the ubiquitylation of RNAPII we performed siRNA mediated silencing of WWP2 and we also used the previously set up experimental system. By using non-targeting siRNA, we could detect the same RNAPII ubiquitylation pattern than it was previously observed. When we used specific siRNA for WWP2 we couldn't detect any changes in the level of ubiquitylated RNAPII compared to the control suggesting an important role of these proteins following DSB damage induced ubiquitylation of RNAPII. To test whether WWP2 was recruited at the site of the damage we performed chromatin immunoprecipitation by using specific antibodies against WWP2. Under normal conditions, we could detect WWP2 at the transcribing unit. 30 minutes after the DSB induction NEDD4 was recruited to the transcribing units, was more robust at the entire damaged gene after break induction. These results indicate that RNAPII is ubiquitylated at the site of the damage by the

E3 ubiquitin ligases recruited to the break site. Additionally, our results demonstrated that the RNAPII degradation was depended on the presence of WWP2 protein.



We showed that WWP2 promoted both NHEJ and RPB1 ubiquitylation at DSBs. However, it was not clear how WWP2 affected the regulation of NHEJ and how this connected to its role in the ubiquitylation of RPB1. It has been known that NHEJ relies on the binding and retention of the heterodimer Ku70/Ku80 from DSB ends allowing the recruitment and activation of DNA-PKcs. To assess how WWP2 affects NHEJ, we first determined the contribution of WWP2 in the accumulation of XRCC4 and Ku80 at DSBs inflicted by UV-A laser micro-irradiation-based live cell imaging. Indeed, depletion of WWP2significantly reduced the recruitment of both core NHEJ proteins. In summary, these findings demonstrate that WWP2 promotes the efficient assembly of NHEJ factors at DSBs, thereby stimulating adequate DNA repair process.



Ubiquitin ligase WWP2 targets RNA polymerase II in a DNA-PK-dependent transcription shutoff circuitry for DNA repair

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Running title: WWP2 targets RNAPII for DNA repair

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