Master thesis project:

Characterization of ribonuclease H enzymes in the *Dictyostelium discoideum* genome

Mobile elements are genomic parasites that amplify as selfish DNA and play important roles in driving the evolution of their hosts. In gene-dense genomes like that of the social amoeba *Dictyostelium discoideum*, mobile elements are confronted with high selection pressure to amplify without causing excessive damage to the host. The targeting of tRNA genes by the *D. discoideum* retrotransposon TRE5-A may have evolved as a means to avoid direct damage to host genes upon retrotransposition. We evaluate how TRE5-A and other *D. discoideum* retrotransposons recognize tRNA genes as integration sites, but we also investigate the regulation of TRE5-A retrotransposition activity.

We constructed a retrotransposition-competent TRE5-A element (TRE5-A^{bsr}) whose integration near tRNA genes can be followed in vivo. The “retrotransposition cassette” *mbsrI* (Siol et al. 2011) used in these assays is based on a blasticidin resistance gene that becomes active only after a complete retrotransposition has occurred. The TRE5-A^{bsr} retrotransposition assay can be used to evaluate the role of host factors in the retrotransposition of TRE5-A (Spaller et al. 2017). This mobile element belongs to the class of non-long terminal repeat retrotransposons and lacks a ribonuclease H (RNase H) domain that is supposed to be required to remove RNA after first-strand cDNA synthesis during the retrotransposition process. Thus, it is in question whether TRE5-A retrotransposition is assisted by cellular RNase H proteins.

Two genes coding for proteins with RNase H2 domains are present in the *D. discoideum* genome. One is characterized as an “archaea-like” RNH, which is thought to act as a monomeric enzyme. The other is a “eucaroyte-like” RNH that is supposed to be active only as a trimer of one catalytic and two regulatory subunits. In this project, we will characterize both cellular RNHs by expressing the respective proteins in bacteria. One aspect of this project is to work out an in vitro assay to determine RNH activity. In particular, we will investigate whether the RNHs have a preference to cut DNA containing a single embedded ribonucleotide, an activity known to initiate removal of ribonucleotides from genomic DNA by a process known as ribonucleotide excision repair. In parallel, we will further characterize the phenotype of previously generated CRISPR/Cas9 mutants of both RNH genes with respect to cellular functions in maintaining genome integrity and the possible involvement in TRE5-A retrotransposition.

O. Siol, T. Spaller, J. Schiefner & T. Winckler (2011). Genetically tagged TRE5-A retrotransposons reveal high amplification rates and authentic target site preference in the Dictyostelium discoideum genome. Nucleic Acids Res. 39(15), 6608-6619

T. Spaller, M. Groth, G. Glöckner & T. Winckler (2017). TRE5-A retrotransposition profiling reveals putative RNA polymerase III transcription complex binding sites on the Dictyostelium extrachromosomal rDNA element. PLoS ONE 12(4): e0175729