Molecular mechanisms underlying the biogenesis of RNA polymerases I and III

In all eukaryotic cells, transcription of RNA from a DNA template is the first step of gene expression and functional RNA synthesis. This process takes place in the nucleus and is carried out by three distinct multi-subunit RNA polymerases (Pols). Whereas Pol II synthesizes messenger RNA and most regulatory RNAs, Pol I is responsible for production of the ribosomal RNA (rRNA) precursor and Pol III synthesizes small non-coding RNA including transfer RNA (tRNA), the 5S rRNA, U6 RNA and others. The molecular mechanisms underlying transcription by all three Pols and their regulation were studied in much detail *in vitro*, *in vivo* and on a structural and functional level over the past decades. However, it remains unclear how the three Pols are assembled, how the assembly is regulated, how Pols are transported into to the nucleus, and whether Pol biosynthesis is common among the three transcription systems. Here, we propose to use the expertise of our groups in studying Pol I and Pol III structure and transcription regulation by teaming up to explore Pol biogenesis. We will identify the sequence and mechanisms of subunit assembly.

We postulate that early Pol I and III assembly steps are interconnected and aim at understanding the basis of the assembly process. A previous study identified conserved eukaryotic protein, Rbs1, which hypothesize to facilitate initial Pol I and Pol III assembly in yeast by a putatively co-translational mechanism. To explore this, we will first use a top-down approach extracting material from living cells to characterize the targeted mRNA-protein and protein-protein interaction of the Rbs1 assembly factor. We will also use a bottom-up approach to recombinantly synthesize Pol assembly intermediates for their structural characterization using cryo-Electron Microscopy (cryo-EM). Finally, we will test our findings in a cell-based system that relies on partially inactivated Pol in a yeast mutant strain in which this transcription system is not essential. Fundamental questions asked in this project are directly linked with a variety of rare human diseases, where mutations of Pols subunits affecting their assembly cause a clinical phenotype, including Treacher-Collins syndrome and Hypomelynating Leukodystrophy.