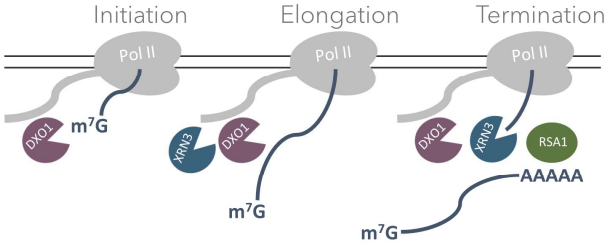
Efficient and properly regulated mechanisms of gene expression underlie the proper functioning of every living cell, and any disruption of these processes leads to the occurrence of a number of disorders, including cancer in humans. Transcription is a particularly important element in the regulation of gene expression as it produces RNA molecules based on the genetic information contained in DNA, and all proteins in the cell, both those that build the cell itself, as well as enzymes and factors that carry out processes that keep the cell alive, are synthesized on the basis of messenger RNA (mRNA). The synthesis of protein-coding mRNAs, as well as many non-coding RNAs with important cellular functions, is carried out by RNA polymerase II, the activity of which is regulated by a number of accessory proteins. Newly-synthesized, nascent RNA molecules also undergo modifications and processing that are crucial for their function. This complex regulation ensures the adaptation of transcription to the current needs of the cell, and enables dynamic changes in gene expression in response to external conditions.

It has long been known that cells organize many of their biochemical reactions in cellular compartments with specific functions. These are not only well-known compartments separated by the cell membrane, such as cell organelles, including nucleus, mitochondria and chloroplasts, but also non-enveloped spaces that are not separated from the inside of the cell by any physical barrier. Recent studies have shown that many of these non-membranous compartments result from phase separation via a mechanism known as liquid-liquid phase separation (LLPS). The LLPS phenomenon makes it possible to organize factors involved in individual cellular processes into clusters, called condensates. The physical proximity of factors that form such condensates enables efficient and properly controlled progress of many biochemical reactions or processes. Notably, the composition of the RNA polymerase II transcription complex that changes dynamically at different stages of transcription is also assisted by LLPS.

The experiments planned in the proposal are aimed at investigating the mechanisms regulating the correct functioning of the transcription cycle in the model plant *Arabidopsis thaliana* and are focused on determining the role of the LLPS in this process. For our research, we chose the XRN3 protein known to be involved in transcription, as well as DXO1 and RSA1, which are functionally connected to XRN3 and have documented or predicted condensate formation properties. To assess the global impact of the proteins of interest on transcription and on the structure of synthesised RNA molecules, we will analyse Arabidopsis mutants lacking these proteins or with their reduced level using integrated next-generation sequencing techniques, including BRB-Seq, ChIP-Seq, RIP-Seq, NET-Seq. We will also investigate the mechanistic bases of the transcription-related functions of XRN3, DXO1 and RSA1 through *in vitro* and *in vivo* analyses of condensate formation by LLPS. We also plan to identify post-translational modifications in these proteins that may mediate LLPS and to describe the influence of external conditions on their transcriptional functions and condensate formation. The obtained results will allow in depth description of XRN3, DXO1 and RSA1 functions in transcription regulation and will establish the role of the LLPS phenomenon in this process. This output will significantly expand the current state of knowledge on the most basic phenomena underlying the functioning of living cells.



- Verification of the involvement of XRN3, DXO1 and RSA1 in transcription
- Identification of factors associated with XRN3, DXO1 and RSA1
- Dissection of the contribution of LLPS to transcriptional regulation
- Impact of post-translational modifications in XRN3, DXO1 and RSA1
- Transcription condensates in plant stress response