Circadian-Regulated Dynamics of Translation in Plants

Mike Haydon, Plant Cell Signalling
University of Melbourne (UoM), Melbourne, Australia (Primary Institution)

Reimo Zoschke, Translational Regulation in Plants
Max Planck Institute of Molecular Plant Physiology (MPI-MP), Potsdam, Germany (Secondary Institution)

Research in the Haydon and Zoschke Labs

The Haydon lab is interested in plant cell signalling, with a particular interest in integration of external and internal signals and their impact on plant physiology. For example, our research has revealed a role for daily rhythms of sugars produced from photosynthesis in setting the pace of the circadian clock in Arabidopsis. This discovery presents an intriguing paradigm for how external (e.g. light) and internal (e.g. metabolism) cues converge to affect plant behaviour. Ongoing research in the lab aims to identify the signalling pathways by which sugars feed into the circadian clock and other regulatory networks. We use genetic and small molecule screens, transcriptomics and molecular genetic tools to better understand the integration of sugar and light signals in plant cells.

In the Zoschke lab we have a research focus on translational regulation in plants. We are fascinated by translation as the interface between RNA and protein metabolism. Our research projects aim at an understanding of the molecular mechanisms of translational regulation in response to in- and external triggers. We use molecular biology, biochemical and genetic approaches to analyse translational regulation, identify the regulatory *cis*-elements and *trans*-factors involved and unravel their molecular mode of action.

Relevant publications from our labs can be found at our webpages.

Project

Background and Project Goals

Circadian clocks evolved in all kingdoms of life to adjust diverse cellular processes to the predictable daily oscillations of external triggers (e.g., light and temperature). These rhythms are driven by a complex regulatory network with multiple layers of transcriptional, translational and post- translational control of gene expression. This complexity enhances robustness of these rhythms, buffering the core oscillator in fluctuating environmental conditions. Two decades of intensive research has defined a core, circadian oscillator in Arabidopsis comprised of multiple, interlocking, so called transcription-translation feedback loops. The primary focus of this research has been on the transcriptional regulation of these components, and to some extent on post-translational control. A critical gap in our knowledge of the circadian network in plants is that of translational control.

Ribosome profiling is a cutting-edge technique that uses next-generation sequencing (NGS) to identify the position and density of ribosomes on mRNAs to calculate the translational efficiency of the transcriptome. This technique has revealed the extent of translational regulation in eukaryotes. For example, there is a pronounced change in translational efficiencies of transcripts in maize and Arabidopsis grown in stress conditions, which is often correlated with differential expression of upstream ORFs (uORFs). We aim to use ribosome profiling to analyse translational dynamics in circadian regulation at a genome-wide scale.

Our goal is (i) to identify transcripts, which are translationally regulated by the circadian clock (i.e., oscillate in translational activity). Additionally, following the circadian dynamics of translational activity will allow us to (ii) define a comprehensive plant translatome and is anticipated to (iii) lead to the discovery of numerous novel ORFs (including upstream and short ORFs), which are only temporally expressed in a limited time window. uORFs are involved in translational regulation of diverse cellular processes. However, only a few uORFs have been assigned to circadian regulation so far. For selected ORFs/uORFs, which exhibit outstanding clock-driven translational regulation, we aim at a molecular understanding of the underlying regulatory mechanisms and the downstream consequences and function of their circadian-regulated translation.

Major Approaches and Timeline

Gene expression has been studied at a transcriptome-wide scale. However, transcription is only the first step of gene expression and much regulation occurs at translational level. Recently, ribosome profiling revolutionized translation analyses by enabling the genome-wide examination of translational regulation at high resolution. Ribosome profiling determines the *in vivo* positions and abundances of ribosomes on mRNAs by deep sequencing RNase-resistant footprints that translating ribosomes leave on their mRNA template. Ribosome profiling has been extensively used to study translational regulation and ribosome behaviour and it currently provides the most comprehensive way to identify translated regions and define ORFs in an unprecedented depth. However, so far its application in plants has been limited. Altogether, ribosome profiling provides an excellent novel entry point into the systematic exploration of translational regulation in circadian oscillations.

Luciferase reporters have proved to be an invaluable tool for the circadian clock field to infer *in vivo* transcriptional activity in Arabidopsis seedlings. The luciferase reporter can also be used as a translational reporter by fusing 5' regulatory sequences in frame with the luciferase gene and/or the target gene coding sequence. By modifying putative elements in the regulatory sequences, such as uORFs, in these constructs by site-directed mutagenesis and transforming into wild-type and mutant seedlings, we can determine the specific contribution of selected regulatory elements for translational regulation and gene function.

Table 1 Timeline of planned work in the PhD project.

	Project aims	1st year	2nd year	3rd year
1.	**Perform time-course, generate reporter constructs**			
	circadian time-course experiment, sample preparation	UoM		
	preparation of translational reporter constructs	UoM		
2.	**Ribosome profiling (RP) and subsequent analyses**			
	ribosome footprint preparation, cDNA library production, NGS		MPI-MP	
	analyses of RP results, identification of novel uORFs		MPI-MP	UoM
3.	**Functional analyses of circadian translation**			
	definition of the circadian translatome		MPI-MP	UoM
	uORF analyses: mutation, complementation of reporter constructs			UoM