SUMMARY

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Investigation and study of deadenylases related to circadian rhythms
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The circadian rhythm is an intrinsic process in all organisms that regulates the sleep-wake cycle and repeats itself almost every 24 hours. They are endogenous, but also adapt to changes in the environment, the most important of which is the light/dark cycles due to Earth's rotation around its axis. Circadian rhythms are driven by the circadian clock, a biochemical mechanism that oscillates with a stable phase and is synchronized with the 24-hour day. Circadian clocks in the body respond to expected environmental changes from the light/dark cycle and adjust accordingly. Thus, rhythms influence various aspects of the physiology and behavior of organisms, such as the sleep cycle, leaf movements in plants, etc. The molecular mechanism of the clock is based on transcriptional regulation, leading to rhythmic changes based on negative feedback loops between transcription and translation controlling the expression of genes involved in the rhythm. Thus, the processes and factors that determine rhythmic gene expression are important for understanding circadian rhythms. Among them, poly(A) tails of RNAs play a key role in stability, translational capacity, as well as degradation. In particular, the shortening and removal of the poly(A) tail by enzymes called deadenylases, is the first and rate limiting step in the degradation of eukaryotic mRNAs, determining their lifespan. Herein, the role of two deadenylases involved in the circadian rhythm, AtHESPERIN and poly(A) specific ribonuclease (PARN) was investigated. HESPERIN was identified from studies from our group as a deadenylase with circadian expression in *Arabidopsis thaliana*. We describe the biochemical characterization of the enzyme through the optimization processes of the production and purification conditions as well as the examination of the active site and the catalytic mechanism. Combining the biochemical results with *in silico* analysis, we assessed the importance of the amino acids required for catalysis, leading to the categorization of HESPERIN in the EEP (exonuclease-endonuclease-phosphatase) family of deadenylases. At the same time, it has been described that PARN exhibits rhythmic mRNA levels in mouse liver, without any other known details about its protein levels or the more general role it may have in the

circadian role. In the context of the thesis, we examined the levels of PARN in human HEK293T cells after synchronization with the circadian rhythm and also in mouse liver. In addition, we examined the interaction of PARN with miR-29a, as studies from our lab have shown that PARN interacts with miR-29a in squamous lung cancer cells and is involved in its maturation, while at the same time it is described that miR-29a plays an important role in period regulation of the clock in mouse liver. Immunoprecipitation of PARN and analysis of RNA molecules bound to it showed that it interacts with miR-29a, followed by precipitation of miR-29a at various time points of 24 hours to identify its protein interactions. Finally, the role of PARN in the circadian rhythm was studied by performing a large-scale proteomic study after immunoprecipitation of PARN from mouse liver over the course of 24 hours. This study revealed novel factors that interact with PARN at specific times of the 24-hour period, but also factors that indicate that PARN may have highly specialized roles within the circadian rhythm, laying the groundwork for the study of these previously unknown processes.