Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis disease (TB), claimed over 1,5 millions of human lives globally only last year. It is one of the primary causes of death to people suffering from immunodeficiencies, like AIDS, and HIV-positive patients accounted for almost 1/3th of those who died from TB last year. Importantly, we observe increasing rates of emergence of drug and multidrug resistance tuberculosis and the scientists worldwide are focusing on finding new generation drugs that may be effective against tuberculosis, looking for protein candidates that may be targeted. It is believed that about 1/3th of the global human population is currently infected with *Mtb*, most cases being the livelong asymptomatic ones, with acute TB onset rates between 5-10%. Currently, there is no drug available to cure the latent form of infection and it may transform into an acute form at virtually any point of person's life, whenever the immune defenses weaken.

Mycobacteria, like any other organisms transcribe and translate their genomic information from DNA into RNA and then into proteins and these processes are critical for continuation of life. The basic principles of mentioned here mechanisms are same between all living cellular organisms, however, some differences can be noted at the molecular level and this gives chance to target bacterial processes separately, without interfering with our cells. Hence, many available drugs interfere with crucial processes, like translation, giving best chance to eradicate the infection. During the lifecycle, an average cell must produce and process a large number of RNA molecules. Their lifespan is very short - in seconds, and any damaged, erroneous or unwanted RNA species must be cleared out from the cell, to enable it to flourish. This is done primarily with the use of RNA degradosome complex. That is also why interfering with RNA decay processes may lead to serious consequences and is often lethal to the cells that lacks the ability to deal with unwanted RNA. The RNA turnover is also critical for adaptation, when global RNA profile rearrangements are necessary to acquire new abilities or functions. Simillar mechanisms are believed to influence the ability of Mtb to switch beteen the dormant, non-replicating form during latent infection and actively dividing form during acute infection.

Recently, central RNA degradosome Rnase - the Rnase E from *Mtb* had been used successfully in a small-molecule target screen and a molecule blocking it has been found as a potential new drug. In the previous study, we have determined the composition of core degradosome components in *Mtb* to be two nucleases that specifically cleave nucleic acids: Rnase E and PNPase; and a helicase that has the ability to unwind RNA - a protein called RhlE. In the proposed project we would like to **determine global substrate specificity of three core RNA degradosome components in *M. tuberculosis*, nucleases: RNAse E and PNPase, and RNA helicase -RhlE. Experiments will be set up to explain their role in overall bacterial physiology and adaptation to oxygen deprivation.**

Understanding the underlying mechanisms that govern RNA stability and turnover is critical to improve our basic knowledge about the pathways of RNA decay but also to target those processes with potential drugs. Here, we propose to determine substrate specificity of the core components of mycobacterial RNA degradosome: Rnase E, PNPase and RhlE, as the major protein complex involved in RNA processing and turnover. We will improve and modify the existing techniques currently applied to study RNA biology and turnover in eukaryotic organisms and optimise them for safe handling with *Mtb*. This project will help to understand the nature and source of mechanisms that are involved in bacterial adaptation via global transcriptome rearrangements. We will collect and evaluate experimental evidence that may be critical for explanation of dormancy phenomenon in mycobacteria. In a long perspective, this will help plan future therapeutic interventions that may result in eradication of billions of latent *Mtb* infections.