# Abstract

**Introduction:**

In recent years, there has been growing interest in regulation of gene expression by small non-coding RNA (sRNA). The first sRNA discovered in 1960s was 6S RNA from *E. coli* (length ~184 nt). It took ~ 30 years to obtain meaningful insights into its function. 6S RNA binds during stationary phase to RNA polymerase (RNAP) containing sigma factor 70 (primary sigma factor), thereby preventing transcription from $\sigma^{70}$ – dependent promoters.

In our laboratory we discovered a small RNA (length ~300 nt) in stationary phase of growht in *Mycobacterium smegmatis*. This sRNA was named Ms 1. The function of Ms 1 is uknown and preliminary experiments indicated that Ms 1may bind to RNAP that lacks σ factor ($\sigma^{A}$).

**Goals:**

The aim of this Diploma project is to contribute to the characterization of Ms 1.

**Approaches:**

First, by molecular cloning, affinity chromatography and *in vitro* transcription I prepared the tools for subsequent experiments *in vitro*: RNAP, $\sigma^{A}$, Ms 1 and its mutated variants. Next, these tools were used for binding experiments on native gels and for transcription experiments.

**Results:**

RNAP, $\sigma^{A}$, Ms 1 and its variants were prepared. *In vitro* binding assays showed that *wt* Ms 1 but not a mutated variant of Ms 1 binds to RNAP. Using this assays were identified areas of Ms 1 that are important for binding.

An active *in vitro* transcription system was established. *M. smegmatis* RNAP containing $\sigma^{A}$ successfully transcribed from the P*veg* promoter.