

ABSTRACT (ENGLISH)

The bacterial cell needs to regulate its gene expression in response to changing environmental conditions. RNA polymerase (RNAP) is the pivotal enzyme of this process and its activity is controlled by a number of auxiliary factors. Here I focus on RNAP-associating factors involved in regulation of transcription in G^+ bacteria: σ factors, initiating nucleoside triphosphates (iNTPs), HelD, δ and small RNA Ms1. The main emphasis is on σ factors from *Bacillus subtilis*.

σ factors allow RNAP to specifically recognize promoter DNA. In my first project I set up *in vitro* transcription systems with purified alternative σ factors, σ^B , σ^D , σ^H , σ^I from *B. subtilis*. Using these systems, I studied the effect of initiating NTP concentration ([iNTP]) on transcription initiation. I showed that promoters of alternative σ factors are often regulated by [iNTP].

In the next project I comprehensively characterized one of the least explored alternative σ factors from *B. subtilis*, σ^I . I identified ~130 genes affected by σ^I , though only 16 of them were directly affected. Moreover, I discovered that σ^I is involved in iron metabolism. Finally, I showed that σ^I binding requires not only the conserved -35 and -10 hexamers, but also extended -35 and -10 elements located in the spacer region.

In collaboration with colleagues-bioinformaticians I studied the gene expression network created for σ^A -regulated genes in *B. subtilis* during spore germination and outgrowth. They predicted new genes to be controlled by σ^A . Using our *in vitro* system I verified the computationally predicted interactions.

Next, I studied δ and HelD, both proteins binding *B. subtilis* RNAP. I showed that δ enhanced transcription with selected σ factors; I demonstrated that HelD had no effect on RNAP affinity for promoter DNA, consistent with findings that HelD affects elongation/termination.

My final contribution was demonstrating that Ms1, a highly abundant sRNA in mycobacteria, has the same transcription start site both in *M. smegmatis* and *M. tuberculosis*, and contributed to the P_{Ms1} promoter characterization.

Together, the results were published in four papers (in two of them I am the first author), advancing our knowledge of transcription regulation in bacteria.