ABSTRACT

Bacterial DNA-dependent RNA polymerase (RNAP) is a key enzyme of bacterial transcription. Its activity must be tightly regulated. This could be done on the level of promoter DNA topology recognition, by changing the intracellular levels of metabolites, or by binding proteins, known as transcription factors.

Even though the RNAP regulatory network has been intensively studied for decades, new regulators are still being described. The main focus of this Thesis is to characterize some of them: i) HelD, a novel RNAP interacting factor, with so far unknown protein 3D structure; ii) RNase J1, an enzyme with a unique mechanism of functioning; iii) Spx, a major regulator of gene expression in *Bacillus subtilis*, with still new roles to be defined and iv) the effect of the topological state of promoters on transcription.

We identified HelD as an interacting protein of RNAP in *Bacillus subtilis* and described its biochemical properties. It stimulates transcription in an ATP-dependent manner, by enhancing recycling of RNAP molecules (Publication I). We published the first insight into the HelD structure by SAXS (small angle X-ray scattering) and deepened the understanding of HelD domain composition (Publication III). And finally, we were able to solve the cryo-EM structure of HelD:RNAP complexes from *Mycobacterium smegmatis* and described the almost whole cycle of HelD in RNAP binding and transcription (Publication VI).

For RNase J1 we described a novel mode of function, not identified in bacteria before, the so-called "torpedo" mechanism, by which RNase J1 helps resolve the RNAPs stalled on DNA (Publication V).

To the already published roles of Spx in various stresses, we add an observation where Spx plays a role in heat stress (Publication II) together with the alarmone ppGpp. Spx and ppGpp were thus newly identified as parts of thermo-tolerance and thermo-resistance in *B. subtilis* (Publication IV).

The last part of the Thesis reveals that the topological state of promoter DNA plays an important role in transcription initiation from primary sigma factor-dependent promoters for ribosomal RNA in various stages of the bacterial growth. It also affects transcription starting from promoters dependent on alternative σ factors (Manuscript VII).

Altogether, this Thesis brings new insights into the functioning of RNAP in particular and the transcription cycle in general.