Poster

# A novel mechanism of bacterial transcription initiation under HelD-mediated protection

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The traditional view of transcription initiation complex formation in *Mycobacterium smegmatis* relies on presence of protein factors σ, RbpA and CarD, assisting RNA polymerase in formation of a functional assembly with promoter DNA. Protein HelD was originally discovered as a partner of bacterial RNA polymerase interfering with transcription. From a series of cryo-EM structures of *M. smegmatis* RNAP-HelD complexes [1] we understood HelD being a protein factor involved mainly in release of stalled transcription complexes, dissociating RNAP from nucleic acids. Recently, the role of HelD has been potentially extended towards a target protection mechanism of antibiotic resistance, namely to rifampicin or closely similar antimycobacterials [2].

While in *B. subtilis* binding of HelD of type I on RNAP excludes concurrent presence of σ, behaviour of type II HelD in *M. smegmatis* differs dramatically. Our recent functional analyses and series of cryo-EM structures bring evidence for HelD from *M. smegmatis* forming complexes with RNAP associated with the primary sigma factor $\sigma^A$ and transcription factor RbpA but also participating in complexes of RNAP with promoter DNA at various stages leading towards initiation complex. The structural snapshots indicate mechanistic aspects of stepwise HelD release from RNAP while enabling formation of transcription initiation complex. Biochemical evidence defines the role of ATP binding and hydrolysis by HelD in the process. The details of interactions of HelD with RNAP, the possible mechanism of rifampicin resistance, of HelD release and of an alternative pathway of transcription initiation will be discussed.

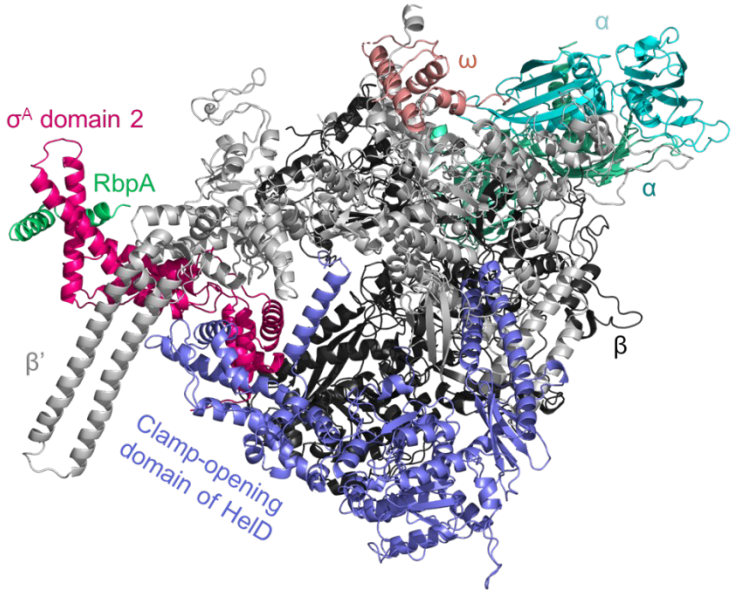


**Figure 1**. Cryo-EM reconstruction at 3.1 Å of *M. smegmatis* holoenzyme with RbpA and HelD. HelD in purple, $\sigma^A$ in hotpink, RbpA in light green.

[1] Kouba, T., Koval', T., Sudzinová, P., Pospíšil, J., Brezovská, B., Hnilicová, J., Šanderová, H., Janoušková, M., Šiková, M., Halada, P., Sýkora, M., Barvík, I., Nováček, J., Trundová, M., Dušková, J., Skálová, T., Chon, U., Murakami, K. S., Dohnálek, J. & Krásný, L. (2020) *Nat. Commun.* **11**, 6419.

[2] Sudzinová, P., Šanderová, H., Koval', T., Skálová, T., Borah, N., Hnilicová, J., Kouba, T., Dohnálek, J., & Krásný, L. (2023). *FEMS Microbiol. Rev.* 47(6), fuac051. https://doi.org/10.1093/femsre/fuac051 Sudzinová, P. *et al.* (2023) *FEMS Microbiol. Rev.* **47**, fuac051.

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