MS081.O02

Structural basis for processive transcription antitermination

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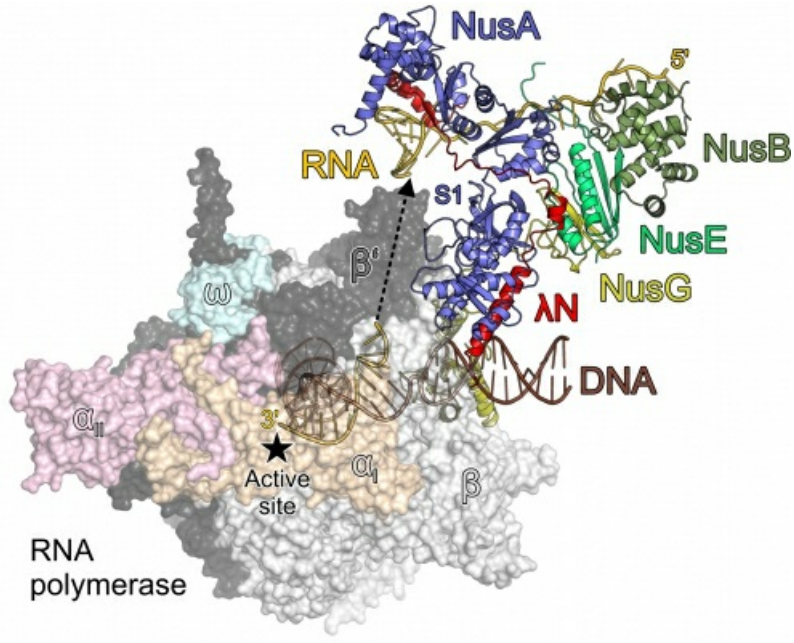
Transcription by RNA polymerase is highly regulated via cis-acting signals on the template DNA and product RNA, as well as via trans-acting protein factors [1]. For example, bacterial RNA polymerase requires a σ factor to specifically initiate transcription at promoter sequences. Furthermore, transcription can be terminated intrinsically via a stable stem-loop structure on the nascent transcript followed by a uridine-rich sequence, or in response to termination factor ρ, an RNA-stimulated ATPase. Transcription is also extensively regulated during the elongation phase, a paradigmatic example of which is afforded by λN-mediated processive transcription antitermination [2]. During λN-mediated antitermination, phage protein λN, host factors NusA, NusB, NusE and NusG and a nut site on nascent RNA cooperate to render RNA polymerase termination-resistant. The structural basis of λN-mediated antitermination has so far remained elusive. We determined a crystal structure of a λN-NusA-NusB-NusE-nut site ribonucleoprotein complex and an electron cryo-microscopic structure of a complete transcription antitermination complex, comprising RNA polymerase, template DNA, product RNA including a nut site, all Nus factors and λN. We validated the modeled organization of the transcription antitermination complex by chemical cross-linking in combination with mass spectrometry. In the λN-NusA-NusB-NusE-nut site complex, the highly extended λN and nut site RNA interconnect NusA, NusB and NusE, forming a triangular assembly. This complex docks via the λN C-terminus and via an N-terminal domain of NusA next to the RNA exit channel on RNA polymerase. The architecture of the transcription antitermination complex suggests multiple mechanisms, by which λN re-programs the host transcription machinery and prevents termination, in agreement with structure-guided functional analyses. (i) The λN C-terminus clamps the RNA exit channel, probably indirectly stabilizing the DNA:RNA hybrid. (ii) λN seems to strategically re-position NusA and RNA-binding elements of RNA polymerase, re-directing nascent RNA and allowing a NusA RNA-binding domain to sequester the upstream branch of a terminator hairpin. (iii) The assembled λN-NusA-NusB-NusE-nut site complex may hinder RNA engagement of termination factor ρ and/or obstruct ρ translocation on the transcript. Our results reveal how, due to intrinsic disorder, λN can act as a multi-protein/RNA interaction hub and thereby mount a multi-pronged strategy to reprogram the Escherichia coli transcriptional machinery.

[1] Zhang, J. & Landick, R. (2016) Trends Biochem Sci 41, 293-310
[2] Nudler, E. & Gottesman, M. E. (2002) Genes Cells 7, 755-768.



Keywords: Bacterial transcription regulation, RNA polymerase, protein λN-dependent antitermination