Local Tuning of the Conformational Flexibility of RfaH

Anna Kahler, Anselm H.C. Horn, Heinrich Sticht

Bioinformatik, Institut für Biochemie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Fahrstraße 17, 91054 Erlangen, Germany

Bacterial gene transcription is regulated by various factors that initiate, elongate, and terminate the transcription process. Elongation factors, such as NusG, interact with the RNA polymerase (RNAP) and prevent the formation of a termination complex at termination sites. NusG is an essential elongation factor in Escherichia coli and is active for almost all genes. The NusG-homologue RfaH, on the other hand, antiterminates transcription via recognition of the *ops* (operon polarity suppressor) element, and therefore elongates the transcript ignoring emerging termination signals. In contrast to NusG, RfaH regulates the transcription of non-essential genes which contain information for different virulence factors, such as haemolysin or lipopolysaccharides.[1]

In the two transcription factors NusG and RfaH the N-terminal domain (NTD) is largely similar and sufficient for full transcription regulation, the different recruitment and activity patterns are caused by distinct structures of the C-terminal domains (CTD): a beta-barrel conformation linked to the NTD in NusG, an alpha-hairpin with hydrophobic contacts to the NTD in RfaH. Dissociation of the two domains in RfaH is thought to be triggered by *ops*-binding and uncovers the RNAP-binding residues on the NTD-surface. Refolding of the alpha-helical CTD into a beta-barrel might lead to interactions of RfaH with translation and secretion machineries.[2]

To gain further insight into domain interactions and stability of the CTD, we performed all-atom molecular dynamics simulations of the crystal structure at different temperatures and salt concentrations and for three double mutants in explicit solvent using the ff99SB force field implemented in AMBER.

Our simulations show that the alpha-helical conformation of the CTD largely fluctuates at 310 K resulting from intrinsic instabilities in the protein sequence. These motions in the CTD – the NTD behaves as a globular protein – can be reduced by lowering the temperature or by increasing the ionic strength. The simulations also revealed that breaking of the helices preferentially occurs at G121 and G125. In silico mutation of these two residues resulted in stabilized CTD-helices suggesting that the flexibility of the two glycines is crucial for the conversion of the alpha-helical CTD into a beta-barrel.

[1] Belogurov, G.A., Mooney, R.A., Svetlov, V., Landick, R. and Artsimovitch, I., Functional specialization of transcription elongation factors, *EMBO J*, **2009**, *28*, 112–122.

[2] Burmann, B.M., Knauer, S.H., Sevostyanova, A., Schweimer, K., Mooney, R.A., Landick, R., Artsimovitch, I. and Rösch, P., An α Helix to β Barrel Domain Switch Transforms the Transcription Factor RfaH into a Translation Factor, *Cell*, **2012**, *150*, 291–303.