Closer to eukaryon: an updated view of the structure of the complete archaeal RNA polymerase

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The archaeal RNA polymerase (RNAP) is structurally and evolutionarily related to eukaryotic RNA Pol II [1] in terms of subunit composition and architecture, promoter elements and basic transcription factors required for Initiation and Elongation.

Obtaining quality diffraction data of crystal of large macromolecular complexes is often a long process made by several optimization steps. Using micro-seeding technique we have obtained a crystal of RNAP from Sulfolobus shibatae (~400kDa) diffracting at 3.2Å. The apparent minimal resolution improvement, from the previous deposited 3.4Å data [2] to the current 3.2Å translates into ~28.000 additional reflections and into a higher signal-to-noise ratio, overall and in the highest resolution shell contributing to a more stable structure refinement. Apart from the visualization of the complete-13 subunit archaeal RNAP structure, the improved electron density has allowed subtle but important structural additions (i) in the large subunit Rpo1, in particular in the clamp-head domain and (ii) of previous un-modelled loops in the Rpo2 subunit. The fully ordered clamp-head domain elucidates the role of sensing-platform for DNA binding. We also revisit the sequence assignment of subunit Rpo13. The position of this subunit proximal to the DNA binding cleft and its helix-turn-helix secondary structure initially suggested a possible interaction with the DNA.

In light of these findings, we have biochemically and biophysically characterized the newly discovered Rpo13 following its expression and purification as a recombinant protein in E.coli. An intriguing gel-filtration elution profile of Rpo13 during purification prompted its characterization by MALDI technique [3]. This analysis uncovered its dimeric form when individually expressed and circular dihroism showed that also in solution ~35% of Rpo13’s residues adopt an unordered structure and infers an intrinsically disordered tendency of this subunit,

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