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

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The archaeal RNA polymerase (RNAP) is structurally and evolutionary 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 MALLS technique [3]. This analysis uncovered its dimeric form when individually expressed and circular dichroism showed that also in solution ~35% of Rpo13´s residues adopt an alpha-helical topology. This result is consistent with the Rpo13 crystal structure and infers an intrinsically disordered tendency of this subunit, a structural property also detected in some eukaryotic transcriptional regulators [4]. Electrophoretic Moblity Shift Assays demonstrate that Rpo13 is able to bind double-stranded DNA in a sequence unspecific manner. These new structural and biophysical data support the proposal that Rpo13 modulates interactions with downstream DNA, conceivably both at initiation and elongation stages. Its presence exemplifies how the ancestral core enzyme was modulated by addition of novel subunits, a process that in eukaryotes has led to the emergence of three different classes of nuclear RNA polymerases [2], [3], [4], [5].

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Transcriptional and translational regulation of cell differentiation

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Cellular differentiation and de-differentiation is regulated on both the transcriptional and translational level. The use of “cocktails” of transcription factors to promote the reprogramming of adult fibroblasts into induced pluripotent stem cells (iPS) has generated tremendous interest in biology and medicine. The originally reported sets of iPS generating factors contained Oct4, Sox2, Klf4 and c-Myc [1] or Oct4, Sox2, Nanog and Lin28 [2]. Here we report on structural and biochemical studies of two of these proteins, Klf4 and Lin28.

Klf4 (Krueppel-like factor 4) is a zinc-finger transcription factor required for the maturation of epithelial tissues. Crystal structure analyses of two different zinc-finger fragments of Klf4 reveal that the two C-terminal C_2H_2 zinc-finger motifs of Klf4 are required for DNA site specificity and the induction of macrophage differentiation [3]. The N-terminal zinc finger, conversely, inhibits the otherwise cryptic self-renewal capacity of Klf4. A Klf4 zinc-finger domain mutant induces self-renewal and block of cell maturation.

Lin28 is a highly conserved RNA-binding protein and was described to modulate the processing of *let-7* microRNA precursors [4]. The small protein contains a cold-shock domain (CSD) and a tandem array of retroviral-type CCHC zinc fingers. Both protein motifs are presumably involved in RNA binding. Crystal structure analysis reveals that the Lin28 CSD resembles the bacterial cold shock proteins. The presence of conserved nucleotide-binding subsites of the surface of Lin28 CSD suggests a common mode of DNA or RNA single-strand binding of Lin28 and bacterial cold shock proteins [5].

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Structural studies of bacterial transcription regulator merr-family protein

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The mercurial compounds are best known for their extreme toxicity to living organisms due to their high affinity towards all thio-containing proteins and a tendency to substitute and block the functions of essential metals. For some bacteria, carrying a suite of