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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

cotranscribed genes, termed the mercury resistance *mer* operon, allows them to survive in environments contain mercurial compounds. The *mer* operon encodes proteins capable of converting inorganic (Hg(II)) and organiomercurial compounds (such as methylmercury, MeHg) to less toxic form (Hg(0)). The *mer* operon transcription is activated by MerR family protein. MerR family protein turns into a transcription activator upon Hg(II) binding. To understand how MerR family protein regulates the transcription of *mer* operon, we have determined the structure of MerR family protein from Gram-positive bacteria *Bacillus megaterium* MB1 by the multiwavelength anomalous diffraction method. The MerR family protein monomer contains a DNA-binding domain, a dimerization helix and a metal-binding motif. Like most other transcription activators, dimerization of MerR family protein is required for function. A total of four MerR family protein dimers are present in the asymmetric unit, all exhibiting similar quaternary structure. The N-terminal DNA-binding domain contains three helices which form a helix-turn-helix motif. The motif is followed by an 8-residue loop and the dimerization domain that is composed of an 8.5-turn alfa-helix. Dimerization of MerR family protein is mediated by packing the two long helices as an antiparallel coiled-coil. The C-terminal metal-binding motif is quite small, consisted of two 3_{10} helices and two connecting residues. A total of four MerR family protein dimers are present in the asymmetric unit, all exhibiting similar quaternary structure. Compared with the structures of other MerR family members, our structure suggests that Hg(II) binding may alter the quaternary structure of MerR family protein. Such a structural transition may reposition the two DNA-binding domains, thus allows the promoter DNA to interact productively with the RNA polymerase to turn on transcription.

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Allostery and disorder mediate transcription regulation by conditional cooperativity

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A common theme observed in the allosteric control of several well-studied transcription factors is the involvement; to some extend, of induced folding of partially unfolded regions. This phenomenon is well established for eukaryotic transcription factors, which frequently possess intrinsically disordered segments or entire domains. Intrinsic disorder is however less well understood for prokaryotes despite the fact that these disordered regions are also predicted in many prokaryotic genomes [1], [2].

Regulation of the *phd/doc* toxin-antitoxin operon involves the toxin Doc as co- or de-repressor depending on the ratio between Phd and Doc. This unexplained transcriptional regulatory phenomenon is known as conditional cooperativity [3]. Binding of Doc to the intrinsically disordered C-terminal domain of Phd structures the DNA binding domain of Phd, for the first time illustrating allostery between two distinct disordered protein domains [4], [5].

In this work we show that a monomeric Doc molecule, capable of interacting with two Phd dimers simultaneously, acts as a bridge between two Phd dimers, increasing the avidity of Phd for DNA and thus enhancing the repression of the operon. Both sites are required for Doc-mediated enhancement of the Phd-operator DNA affinity. Moreover our studies on Phd provide for the first time direct experimental evidence demonstrating allosteric coupling between two (partially) disordered domains. This coupling is disrupted in certain mutants of Phd that nevertheless retain wild type like binding of Doc. The N-terminal domain of Phd exists in solution as an equilibrium between a DNA binding-competent ordered state and a DNA binding-incompetent disordered state. The equilibrium between both states is influenced not only by its direct ligand, the operator site, but also by binding of the Doc co-repressor to the intrinsically disordered C-terminal segment of Phd. Our combined structural and biochemical data allow us to put forward a model for the regulation of the *phd/doc* operon that explains conditional cooperativity [5].

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Structural basis for DNA recognition and binding specificity by the transcription factor Ets2

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The transcription factor Ets2 is a member of the Ets transcription factor family, which regulates target gene transcriptions during various cellular events, such as differentiation, proliferation, senescence and oncogenic transformation. All the family members bear a highly conserved DNA binding domain, termed the Ets domain (ETSD), and recognize the consensus sequence containing 5'-GGA/T-3' and additional flanking sites. While each Ets family member recognizes the specific target promoter and binding site, how the precise machinery discriminates the specific target promoter sequence is poorly understood. Here we show the molecular details of the DNA recognition machinery, Ets2. To clarify the molecular details of DNA binding mechanisms, we determined a 3.0 Å crystal structure of the Ets2ETSD in complex with the target DNA. Ets2 recognized the target DNA via almost similar mechanism, as did Ets1. Furthermore, to evaluate that the additional flanking sequences around consensus binding site affect DNA binding affinity of Ets2, we measured kinetic parameters of interactions between Ets2ETSD and various Ets2 target sequences by the SPR method. These showed that additional flanking sequences around the consensus sequence and base-base stacking energy of the respective target sequence provided target promoter specificity and selectivity by Ets2. Additionally, we performed Luciferase assay to estimate that these physicochemical properties for DNA binding attribute cellular transcriptional activities. This indicated that down-regulation of the Ets2 transactivation activity was correlated to reducing DNA binding affinity of Ets2. Our results demonstrate that DNA binding affinity derived from both of amino acid sequence of transcription factor and target promoter sequence affects subsequent transcriptional regulation activity.

Keywords: Transcription factor, DNA-protein complex structure, DNA recognition