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The closed structure of an archaeal DNA ligase from *Pyrococcus furiosus*

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DNA ligases join single-strand breaks in double-stranded DNA, and are essential to maintain genome integrity in DNA metabolism. Here, we report the 1.8 Å resolution structure of *Pyrococcus furiosus* DNA ligase (PfuLig). The enzyme comprises the N-terminal DNA binding domain, the middle adenylation domain, and the C-terminal OB-fold domain. The architecture of each domain resembles those of human DNA ligase I, but the domain arrangements strikingly differ between the two enzymes. The closed conformation of the two "catalytic core" domains at the carboxyl terminus in PfuLig creates a small compartment, which holds a non-covalently bound AMP. This domain rearrangement results from the "domain-connecting" role of the helical extension conserved at the C-termini in archaeal and eukaryotic DNA ligases. The DNA substrate in the human open-ligase is replaced by motif VI in the Pfu closed-ligase. Both the shapes and electrostatic distributions are similar between motif VI and the DNA substrate, suggesting that motif VI in the closed state mimics the incoming substrate DNA. Two basic residues (R531 and K534) in motif VI reside within the active site pocket and interact with the phosphate group of the bound AMP. The crystallographic and functional analyses of mutant enzymes revealed that these two residues within the RxDK sequence play essential and complementary roles in ATP processing.

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Classification of Protein-DNA Complexes Based on Structural Descriptors

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DNA-binding proteins are usually classified according to the structural motif of proteins. However, the relationship between such classification and the mechanism of DNA sequence recognition is poorly understood. The conservation of structural motif in proteins may not necessarily dictate the way by which DNA sequences are recognized. We attempt to classify protein-DNA complexes using various descriptors, several of them characterizing protein-DNA interactions, in contrast with the conventional motif-based classification. The cluster analyses were carried out for a unique set of 62 complexes including a variety of protein motifs, and 7 distinct clusters were revealed from the analyses. We found that some proteins with the same motif are classified into different clusters whereas different proteins with distinct motifs are classified into the same cluster. Thus, these results suggest that the motif-based classification of DNA-binding proteins may not necessarily correspond to structural and functional properties characterizing protein-DNA recognition, and that the present classification will help to identify common properties and rules that govern protein-DNA recognition.

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Free Energy Landscape for Interactions between DNA Bases and Protein Side Chains

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To understand the origin of the specificity in protein-DNA recognition, we calculated the free energy ($\Delta\Delta G$), enthalpy ($\Delta\Delta H$), entropy ($T\Delta\Delta S$) and minimum interaction energy ($\Delta\Delta E_{\text{min}}$) for interactions between an amino acid side chain and a base pair (AT or GC) by means of the systematic sampling. We presented them as maps on the base pair plane. We also calculated $\Delta\Delta G$ maps between an amino acid side chain and 32 kinds of stacked three base pairs. By utilizing thus calculated $\Delta\Delta G$ maps, we can construct $\Delta\Delta G$ isosurfaces for any DNA sequences. Some areas are found with lower $\Delta\Delta G$ values discretely along the major groove of DNA sequences. We demonstrate that the predicted lower $\Delta\Delta G$ value regions are consistent with the regions where amino acid side chains are involved in direct contact with DNA bases within protein-DNA complexes in Protein Data Bank (PDB). We also present the comparison between the systematic sampling and the multicanonical Monte Carlo sampling methods to generate interaction configurations between base pairs and a protein side chain.

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Possible contradiction of the consensus mechanism of *E. coli* sigma-70 and the observed activity of its N-terminus half proteins

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Bacterial sigma subunit of RNAP is essentially required to initiate transcription from promoters. In *E. coli*, the σ^{70} subunit is responsible for vegetative transcription and involves Region 1~4, the domains conserved in other sigmas of *E. coli* and in other species. The σ^{70} is believed to function by using these regions for binding to promoters and to other subunits of RNAP, the conventional model suggested from many genetic and biochemical studies. We have constructed a disruptant strain of *rpoD*, the gene encoding essential σ^{70} , which is maintained by an *rpoD* allele on a plasmid. The allele encoding GFP protein sandwiched with Region 1 and Region 2-4 of σ^{70} maintained the disruptant. From the strain we purified RNAP holoenzyme, and the preparation was active on transcription from T7A1 promoter. Notably we found the GFP-fused sigma had been cleaved at the middle of Region 2, if judged from the N-terminus peptide sequencing and MS, and no residual C-terminal fragments of σ^{70} were found in the enzyme preparation. The missing regions contain the latter half of Region 2.4 that recognizes -10 promoter, Region 3 that forms the binding site of NTP and fills the RNA exit channel, and Region 4 that recognizes -35 promoter. As expected, our tentative experiment shows the plasmid harboring the truncated GFP- σ^{70} did not maintain the *rpoD* disruptant, suggesting that a small amount of uncleaved GFP- σ^{70} protein complements the lack of σ^{70} . However, it is fascinating to clarify the mechanism of transcription from T7A1 promoter by the truncated σ^{70} .