Macromolecular Assemblies

MS04.08.01 DNA RECOGNITION BY PROTEIN COMPLEXES DURING EUKARYOTIC TRANSCRIPTION INITIATION. Stephen K. Burley, The Rockefeller University and Howard Hughes Medical Institute, New York, New York, USA

The TATA box-binding protein (TBP) is required by all three eukaryotic RNA polymerases for correct initiation of transcription of ribosomal, messenger, small nuclear and transfer RNAs. In the most general case, pol II transcription of mRNA begins with TBP-mediated recognition of a TATA box located immediately upstream of the transcription start site. The structure of a TBP from *Arabidopsis thaliana* complexed with a fourteen base pair oligonucleotide bearing the Adenovirus major late promoter (AdMLP) TATA element has been determined at 1.9Å resolution. Binding of the monomeric, saddle-shaped protein induces an unprecedented conformational change in the DNA. Insertion of two pairs of phenylalanine side chains into two base steps (TpA and ApG) produces two sharp kinks at either end of the sequence TATAAAAG. Between the kinks the double helix is partially unwound and smoothly bent, approximating the widened minor groove face of the TATA element to the concave surface of the molecular saddle. More recently, the structure of a ternary complex of transcription factor IIB (TFIIB), TBP, and the AdMLP TATA element has been determined at 2.7Å resolution. The C-terminal/core region of TFIIB consists of two quasi-identical helical domains, separated by a cleft that grasps TBP's acidic C-terminal stirrup. The structure of the TBP-DNA complex is essentially unaffected by contact with the basic surface of core TFIIB, which also interacts with the phospho-ribose backbone up- and downstream of the center of the TATA element. The N-terminal domain of core TFIIB is located on the downstream surface of the ternary complex, where it could interact with RNA polymerase II and help fix the transcription start site. The upper surface and N-terminal stirrup of TBP, and the remaining surfaces of core TFIIB are available for interactions with TBP-associated factors, other class II initiation factors, and transcriptional activators and coactivators.

MS04.08.02 INITIAL PHASING IN RIBOSOMAL CRYSTALLOGRAPHY. Z. Berkovitch-Yellin[1], I. Agmon[1], K. Anagnostopoulos[2,3], H. Bartels[2], A. Bashan[1], W.S. Bennett[2], A.Dribin[1], F. Franceschi[3], H.A.S. Hansen[2], J. Harms[2], S. Krumbholz[2], I. Levin[1], S. Morlang[3], M. Peretz[1], I. Sagi[1,3], F. Schlunzen[2],R. Sharon[1], J. Thygesen[2], A. Tocilj[1], N. Volkmann[2], O. Weinberg[1], S. Weinstein[1] & A. Yonath[1,2]. [1]Struc. Biol., Weizmann Inst., Rehovot, Israel, [2]MPG Lab. for Ribosomal Struc., Hamburg, Germany, and Max-Planck-Inst. for Molecular Genetics, Berlin, Germany[3]

The universal cell organelles facilitating the process protein biosynthesis are nucleoprotein assemblies, the ribosomes. A typical bacterial ribosome weighs over 2.3 million daltons and contains 57-73 different proteins and 3 RNA chains of about 4500 nucleotides, arranged in two subunits of unequal size. For illuminating the detailed mechanism of the translation of the genetic code into polypeptide chains, we have initiated crystallographic studies.

Diffracting crystals have been grown from ribosomes and their complexes with nonribosomal components participating in protein biosynthesis, as well as from native, chemically modified and mutated ribosomal subunits. Those that diffract to the highest resolution obtained so far for ribosomal crystals, 2.9 Å, are of the large ribosomal subunits from Haloarcula marismortui. X-ray data are being collected with bright synchrotron radiation at cryogenic temperatures from flash-frozen crystals. For phasing by isomorphous replacement methods, heavy atom derivatization is being performed either by soaking crystals in solution of heteropolyanions and multi-metal coordination compounds, or by specific and covalent attachment of monofunctional reagents of dense organo-metalo clusters prior to the crystallization.

The suitability of both approaches for phasing at intermediate resolution has been clearly demonstrated in the construction of the following: a 9 Å and 20 Å SIR electron density maps of tbe large ribosomal subunits (of halophilic and mesothermophilic bacteria, respectively); and a 16 Å MIR map of the small ribosomal subunits from thermophilic bacteria. The resulting maps show features of size similar to that expected for this particle, and could be further refined by solvent flattening.

Aiming at phasing at higher resolution as well as at assisting the interpretation of the electron density map, procedures for specific derivatization with rather small and compact clusters are being developed. As there are no exposed cysteines suitable for cluster binding on the surface of the halophilic ribosomes, these are being inserted by site: directed mutagenesis. The corresponding genes are being isolated, sequenced and mutated in positions determined by surface-mapping experiments. In addition, two internal complexes, composed of proteins and rRNA fragments have been isolated. These are being produced for crystallographic analysis.

MS04.08.03 THE CRYSTALLOGRAPHIC STRUCTURE OF A G PROTEIN HETEROTRIMER. Stephen R. Sprang, Mark A. Wall, David E. Coleman, Ethan Lee, Jorge A. Iniguez-Lluhi, Bruce A. Posner, Alfred G. Gilman, Departments of Biochemistry and Pharmacology, Howard Hughes Medcial Institute, University of Texas Southwestern Medical Center, Dallas, TX, 75235, USA

We have determined the three-dimensional structure of the signal transduction complex comprising a heterotrimer of the GDP-bound α(Gi-α), β-1 and γ-2 subunits at a resolution of 2.4 Å. In this state, the α subunit and the β-γ dimer are maintained in a non-signalling complex. Activated, heptahelical receptors promote the disassociation of this heterotrimeric complex by triggering the release of GDP from the α subunit. The structure reveals two nonoverlapping regions of contact between α and β, an extended interface between β and nearly all of γ, and limited interaction of α with γ. The major α/β interface covers a flexible helix (switch II) in the α subunit which adopts different conformations in the GDP and GTP-bound states. The β subunit stabilizes a conformation of the switch II helix that traps GDP in the binding site of the α subunit. The α-β interface contains a large, negatively charged surface that may offer electrostatic complementarity to the cytoplasmic domains of the activated receptor. The helical amino terminus of the α subunit forms a second region of contact with the "side" of the β subunit. Repeated "WD" motifs in β form a circularized seven-fold β propeller, which has been observed in a variety of unrelated proteins. In the WD family, the conserved cores of these motifs may serve as scaffolds for display of variable linkers on the exterior face of each propeller blade. The structure was determined by a combination of MIR and molecular replacement methods and has been refined to an R-factor of 0.24 (R_{free}=.32).