most recent studies and unpublished data. In particular, I will present our current model for transcription initiation at a gene promoter [1], the most recent insights into transcription elongation, in particular how RNA polymerase II can arrest and then be reactivated [2], and how the only universally conserved transcription factor, Spt5 (or NusG in bacteria), prevents premature termination by enclosing the DNA and rendering transcription processive [3].

[1] D. Kostrewa et al., P. Cramer *Nature,* **2009**, *462*, 323. [2] A.C.M. Cheung, P. Cramer *Nature* **2011**. [3] F.W. Martinez-Rucobo et al., P. Cramer *EMBO J.* **2011,** *8 March.*

Keywords: gene transcription, RNA polymearse, multiprotein complex

MS.78.2

Crystal structures of bacterial and yeast ribosomes

Marat Yusupov, Adam Ben-Shem, Lasse Jenner, Natalia Demeshkina and Gulnara Yusupova. *Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch (France).* E-mail: marat@igbmc.fr

The crystal structure of the yeast 80S ribosome determined at 4.15 Å resolution reveals the higher complexity of eukaryotic ribosomes, which are 40% larger than their bacterial counterparts [1]. Our crystals capture the ribosome in the ratcheted state which is essential for translocation of mRNA and tRNA and where the small ribosomal subunit has rotated with respect to the large subunit. We describe the conformational changes in both ribosomal subunits that are involved in ratcheting, and their implications to mRNA and tRNA translocation. Structural rearrangements of the ribosome in the tRNA binding step have been studied on bacterial ribosome model. Discrimination of tRNA on the ribosome occurs in two consecutive steps: initial selection and proofreading. We propose a proofreading mechanism based on comparison of crystal structures of the 70S ribosome with an empty A site or the A site occupied by cognate or non-cognate tRNA [2]. We have shown involvement of tales of ribosomal proteins in stabilization of correct tRNA on the ribosome. We suggest that proofreading begins with stabilization of tRNA anticodon loop with involvement of magnesium ions, following by stabilization of elbow region and accommodation of the acceptor end in the peptidyl transferase center.

[1] 1. Ben-Shem, A., Jenner, L., Yusupova, G., Yusupov, M. *Science,* **2010**, 330, 1203-1209. [2] Jenner L, Demeshkina N, Yusupova G , Yusupov M. *Nat. Struct. Mol. Biol.* **2010**, 17, 1072-1078.

Keywords: yeast ribosome structure, x-ray, proofreading

MS.78.3

The *GAL* regulon in *S. CEREVISIAE*: the Gal3p/Gal80p interaction

Tali Lavy, Rajesh K. Prakash, Hongzhen He and Leemor Joshua-Tor, *Howard Hughes Medical Institute and Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor (USA), NY 11724*

S. cerevisiae responds to galactose by activating the coordinated transcription of a family of genes known as the *GAL* genes. This transcription is regulated by the *GAL* regulatory switch, which consists of three key protein components: Gal4p, the transcriptional activator; Gal80p, the repressor; and Gal3p, the transducer. The interplay between Gal3p, Gal80p and Gal4p determines the on/off status of the switch. Gal4p binds the upstream activating sequences of *GAL* genes (UAS$_{gal}$) through its N-terminal domain and activates transcription through interaction of its C-terminal transcription activation domain (TAD) with the transcription machinery. During non-inducing conditions, Gal80p binds to the TAD of Gal4p to repress transcription. Repression is relieved upon induction with galactose, mediated by the transducer, Gal3p. We have shown previously that NADP also plays a role in activating the switch [1]. Gal3p forms a complex with Gal80p, α-D-galactose and ATP.

We have solved the crystal structure of the tertiary complex of Gal80p-Gal3p with α-D-galactose and ATP to 2.1Å resolution. The structure shows that the interaction between Gal3p and Gal80p occurs only when Gal3p is in a "closed conformation" in which the N- and C- terminal domains of the protein are closer together. This conformation is induced by α-D-galactose and ATP binding. The Gal3p interaction surface of Gal80p is different from the surface for Gal4p TAD interaction and the structure reveals the basis for super-repressor phenotypes of Gal80p. Gal3p is 75% identical in sequence to Gal1p, but lacks the galactokinase activity of the Gal1p enzyme. We show that the α-D-galactose conformation found in our structure is unique compared to what is found in other galactokinase structures and might be the basis for the lack of galactokinase activity in Gal3p.

[1] Kumar, Rajesh P., *et al.* , *Science* **2008,** *319,* 1090-1092.

Keywords: eukaryotic transcription, GAL regulon

MS.78.4

Structure and function of human BMAL1-CLOCK-DNA complex

Zixi Wang, Xiao-Dong Su, *State Key Laboratory of Protein and Plant Gene Research, School of Life Sciences, Peking University, Beijing 100871, (China).* E-mail: xdsu@pku.edu.cn

The molecular mechanism of circadian rhythm from flies to human is evolutionarily conserved as an autonomic oscillator with a transcription activation - inhibition cycle of approximately 24 hours. In human, the circadian transcription control process is regulated by two basic helix-loop-helix (bHLH) PAS domain containing transcription factors, namely BMAL1 and CLOCK as the master controller. The BMAL1/CLOCK complex is proved biochemically to form heterodimers to bind the E-box DNA sequence CACGTG to accomplish their functions.

We present here the crystal structure of BMAL1/CLOCK bHLH domains in complex with DNA at 2.4 Å resolution. The current refined R_{work} and R_{free} values are 24.5% and 29.6%, respectively. The basic regions helices of BMAL1 and CLOCK insert into the major groove of DNA, with the H77, E81, R85 in the BMAL1 and R39, E43 and R37 in the CLOCK as the recognition residues to the E-box sequence CACGTG. The major interactions between these residues and base pairs are hydrogen bonds. The helix after the basic region and the helix after the loop region of BMAL1 and CLOCK form a left handed helices bundle, which contribute to the interaction and recognition region of heterodimer. The heterodimer interface is mainly stabilized by two layers of hydrophobic interactions. Each layer contains four residues, forming the knobs into holes packing. If a BMAL1-BMAL1 homodimer or a CLOCK-CLOCK homodimer model was generated, a series of side chain clashes would be observed. In addition, we observed a dimer of heterodimer in the crystal packing, with the interface between two heterodimers fully mediated by BMAL1. This tetramer form was also confirmed by biochemical and biophysical means with strong functional implications. The two DNA chains in the tetramer