most recent studies and unpublished data.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].


Keywords: gene transcription, RNA polymerase, multiprotein complex

MS.78.2


Crystal structures of bacterial and yeast ribosomes

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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 observed involvement of roles 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.


Keywords: yeast ribosome structure, x-ray, proofreading

MS.78.3


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

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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 (UASα) 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.


Keywords: eukaryotic transcription, GAL regulon

MS.78.4


Structure and function of human BMAL1-CLOCK-DNA complex

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The molecular mechanism of circadian rhythm from flies to human is evolutionarily conserved as an autonomous 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 Rmin and Rmax 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...
form an angle of 120 degrees due to the crystallographic symmetry, indicating a possible DNA looping mechanism during transcription activation and inhibition.

Keywords: crystal_structure, circadian_rhythm, transcription_control

MS.78.5


Crystal structure analysis of release factor 3
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Translation of an mRNA is terminated when a stop codon is encountered. Since tRNAs do not recognize stop codons, this event is performed by proteins called release factors. Prokaryotes have two class I release factors (RFs), RF1 and RF2, and one class II release factor, the G protein RF3. RF1 and RF2 hydrolyze and release the completed polypeptide from the peptidyl-tRNA at the ribosomal P-site in response to a stop codon. RF3 binds to the ribosome to promote rapid dissociation of RF1 or RF2 from the A-site in a GTP-dependent manner. We have studied the structure-function relationship of the RF3 from sulfate-reducing bacterium, Desulfovibrio vulgaris Miyazaki F. Here we present the high resolution crystal structures of RF3 complexed with GDP and guanosine 3'5'-bis(diphosphate) (ppGpp).

ppGpp is known as an alarmone which is involved in stringent response in bacteria. In cells growing under optimal conditions, the concentration of GDP is much dominant over that of ppGpp. Under stress conditions, however, the concentration of ppGpp increases strikingly, and attains levels over that of GDP. In the structure of RF3 complexed with ppGpp, ppGpp binds at the same nucleotide-binding site in an almost identical manner with GDP, suggesting that GDP and ppGpp are two alternative physiologically relevant ligands to RF3. We have found that ppGpp blocks the recycling of RF1 or RF2 by RF3 in bacterial ribosome. It is probably because ppGpp interferes either binding of RF3 to ribosome or replacement of GDP by GTP in the RF3 ribosome complex. These lines of evidences suggest that RF3 would have functions of a cellular metabolic sensor and/or regulator that switches between the active GDP-bound form which allows active protein syntheses under the normal condition and the low-active ppGpp-bound form when shortage of nutrients are detrimental.

Keywords: structure_function_relationship, stress, translation_factor

MS.79.1


Electron crystallography: harder, better, faster, stronger
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The stronger interaction of electrons with matter allows nanoscale crystals to be investigated, which is of increasing importance, academically and commercially, given the growth of nanotechnology research and application during the last decade. Electron crystallography fills the void between crystals which are too small for single crystal X-ray studies and powder diffraction experiments that fail to yield a structure. However, it is often viewed as a technically difficult and time-consuming method for structure solution.

The development of the Automated Diffraction Tomography (ADT) [1] for electron crystallography has led to a number of distinct advantages over traditional methods, in terms of data collection, quality, quantity and the ability to solve structures ab initio via direct methods. The most appealing advantage, when compared to traditional approaches which require zonal diffraction data and complementary real space image, is the vastly reduced time it takes to solve a structure from diffraction intensities only, in favourable cases data collection and structure solution can be completed within a single day.

The details of how ADT methodology performs data collection from nanocrystals shall be outlined. The new data collection geometry has some striking advantages, it provides a vastly improved coverage of reciprocal space, when compared to zone axis data sets, as well as reduced dynamical effects. The new processing requirements [2], for extracting the intensities for structure solution, these will be discussed in detail.

The improvements in data quality gained by using ADT for electron crystallography have two distinct advantages. 1. Direct Methods can be used routinely for structure solution [3], 2. Structures more difficult and complex can be solved by electron diffraction data alone than previously thought possible. Examples of structures solved using ADT, which were not possible by any other method, will be presented.

We hope to convey the benefits of using the ADT approach for electron crystallography and appeal to crystallographers who would not normally consider using electron crystallography that it may be a viable approach to consider in the future for solving problematic nanocrystals which would not yield an answer to their tried and trusted methods.


Keywords: automated diffraction tomography, nanocrystal, software

MS.79.2


Modulated structures and TEM’s: from relaxor ferroelectrics to nano-chessboards
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Functionally useful materials are often modulated and frequently inherently flexible [1] i.e. materials whose local structures and properties are finely balanced and hence able to respond to the application of external signals e.g. electric and/or magnetic fields, strains, changes in temperature, composition etc. Materials of this type (piezoelectrics, relaxor ferroelectrics, ionic conductors, solid solutions etc.) are ubiquitous in devices all around us e.g. mobile phones, sensors, solid oxide fuel cells. A detailed understanding of structure, both average as well as local (on the relevant length and time scales, see e.g. Fig.1 below) of such materials is essential for an understanding of their properties and of methods to optimize and manipulate them.