DNA. Constructs of segments of the α subunit show that the N-terminal region is responsible for dsDNA stabilization, while the C-terminal region binds to melted DNA.

272.13-Pos Direct Observation of Bacteriophage Mu Target Immunity with Single-Molecule Analysis

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Board B105.13

Transposons are mobile genetic elements that transfer DNA from one site within a genome to another site. Bacteriophage Mu is one of the transposon family members and the mechanism of DNA transposition is well-studied. Highly specific protein-DNA complexes are required for the DNA cleavages and joining involved in DNA transposition. MuA is the transposase, which binds to the Transposase binding sites at each end of the Mu genome and then assembles into a series of nucleoprotein complexes called transpososomes within which the DNA cutting and joining reactions take place. Another Mu encoded protein MuB is an ATP-dependent nonspecific DNA binding protein and in the presence of ATP, assembles into large oligomeric complexes on DNA, which serves as an efficient transposition target site. MuA stimulates ATPase of MuB, resulting in dissociation of MuB from DNA. Therefore, MuB accumulates on DNA that is not bound by MuA, resulting in a strong preference for transposition to occur at DNA sites at least 10 kb from MuA-bound regions. This phenomenon is called target immunity and thought to prevent destruction of the Mu genome by auto-integration. Although the detailed mechanism of target immunity still remains to be studied, recent analysis using enhanced green fluorescent protein (EGFP)-MuB shows that MuB oligomers accumulate at A/T-rich sequences and DNA looping between the MuA- and MuB-bound DNA sites is required for MuA-stimulated removal of MuB from DNA. In this study, we constructed fluorescent labeled MuA-DNA complex and used single-molecule fluorescence detection system to clarify the target immunity mechanism in more detail. We will show our present results and discuss the mechanism of target immunity.

272.14-Pos Insights in the transposition mechanism of the bacterial insertion sequence IS*911* revealed by Tethered Particle Motion

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Board B105.14

During the transposition process, the DNA segments called insertion sequences move between different loci in bacterial genomes. We focus on IS*911* a member of the widespread IS3 family of bacterial insertion sequences which follows a two-step transposition mechanism involving the formation of a circular transposon intermediate. IS*911* encodes itself for the transposase OrfAB responsible for this process and, like other insertion sequences, IS*911* is bordered by short imperfectly repeated sequences, IRL and IRR, in an inverted orientation. These are essential for the productive transposition, since they provide the specificity for both the binding of the OrfAB and the cleavage and strand transfer reactions required for the displacement of the element.

We follow the assembly of the synaptic complex occurring during this transposition process by using the single molecule techniques of Tethered Particle Motion which consists in tracking the movement of a bead tethered by a DNA molecule to the glass surface. It enables us to monitor the various protein-mediated looping states of the DNA consecutives to the interaction with the active transposase mutant OrfAB4M. New states are observed when compared to the results obtained with the inactive truncated form of the transposase OrfAB[149]. Moreover preliminary results show that in absence of Mg2+ the synaptic complex cannot be formed with the OrfAB4M. Additional experiments are in progress in order to elucidate these recent and interesting findings.

Ribosome & Translation

273-Pos Simulation of an Entire Cycle of tRNA Translocation of Ribosome Using Hybrid Elastic Network Interpolation

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Board B106

We first simulate the complete cycle of tRNA translocation during ratcheting motion of ribosome using hybrid elastic network interpolation (HENI) in which both rigid and flexible regions of a complex system can be represented as a spring network among rigid clusters and point masses. A 70S ribosome (2HGP and 2HGQ) structure was modeled as four rigid clusters (30S head, 30S body, L1 stalk, and 50S body) and point masses including all three tRNAs, mRNA, and coarse-grained residues or nucleotides nearby the interface region between 30S and 50S subunits. By adopting Frank's two-step mechanism for translocation (Nature, 2000), we applied group theory to compute the coordinates of hybrid and posttranslocation states of ribosome, respectively. HENI result for the first step from pretranslocation state to hybrid state demonstrates how A(P) site tRNA translocates from A(P) site to P(E) site of 50S subunit. Similarly, it is also observed that 30S subunit returns to the original position with respect to 50S subunit by relatively shifting A(P) site tRNA to P(E) site during the second step. Overlap calculation based on normal mode analysis of the same ribosome structure indicates not only that ratcheting motion is the most dominant mode during the cycle of tRNA translocation but also that other associated motions such as 30S head rotation, A site opening, and E site opening are crucial for explaining how mechanically tRNAs cross the bulge regions between A(P) and P(E) sites.

274-Pos The A/T (Pre-accommodated) State Observed with Phe-tRNA^{Phe}, Trp-tRNA^{Trp}, and Leu-tRNA^{Leu}

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Transfer RNA enters the ribosome in a distorted, high-energy form [1]. The anticodon arm is kinked and twisted compared to the X-ray structure. This conformation was observed by cryo-EM in a complex of the *E. coli* ribosome with Phe-tRNA^{Phe}•EF-Tu•GDP• kirromycin and UUU at the A site [1]. The map showed aa-tRNA before accommodation and led to the conclusion that accommodation into the A site might occur similar to the relaxation of a spring. The result was thought to have implications for the nature of the conformational signal from the decoding center to the large subunit. To date, however, the hypothesis that this mechanism holds for *all* aminoacyl-tRNAs has not been tested.

We describe here two additional studies, one with UGG at the A site and cognate Trp-tRNA^{Trp}, the other with CUG at the A site and cognate Leu-tRNA^{Leu}, again stalled with kirromycin after GTP hydrolysis. Fitting the cryo-EM maps (for Phe, Trp, and Leu) with atomic models using real-space refinement [2] shows:

1. the CCA end, as well as the EF-Tu domain associated with it, is located at a conserved position;
2. nt56 in the T-loop of the A/T-tRNA and the A1067 in the GAC are always connected even though this position is variable among the three maps;
3. the positions of the D-loop in the three maps vary widely, and none is associated with the T-loop as in the X-ray structures; and
4. the anticodon arm distortion [1] is a common feature. Additionally, the variable loop of Leu-tRNA^{Leu}, which is unusually extended, makes a separate contact with the beak of the small subunit, which may assist in the selection of this tRNA.

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275-Pos Molecular Dynamics Simulations Of Nascent Peptide Chains In The Ribosomal Exit Tunnel

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Board B108

Nascent peptide chains are polymerized by the ribosome and exit through a tunnel in the large subunit, which stretches for about 10 nm from peptidyl transferase center and to the opposite side of the subunit [1]. The nascent polypeptide SecM causes a translation arrest after the peptide link formation of Pro166 deactivating the ribosome until the arrest is canceled [2].

To gain insight into the mechanism of the arrest, extensive molecular dynamics simulations with the SecM peptide at the arrest point, two amino acids before the arrest point, and of the Bpp peptide were performed. The simulation system contained the large ribosomal subunit, the nascent peptide, and explicit water. Principal component analysis of the polypeptide motion suggest differential dynamics for the different peptides. The final structures of the peptides assume different conformations inside the tunnel. The backbone rmsd of the end structures of the arresting SecM and the non-arresting Bpp peptide is 0.65 nm.

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276-Pos Functional Ribosome Complex at 6.7Å Resolution: Conformational Changes in EF-Tu Upon GTP Hydrolysis

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We have explored the influence of a number of parameters in image processing of a ribosome data set with the aim to substantially improve the resolution the 3D density map. This study has resulted in a 6.7-Å map of the *E. coli* ribosome for a complex identical with one used previously for a 10.25Å cryo-EM map (1).

Micrographs were recorded at low-dose conditions on an FEI Tecnai F30 Polara electron microscope operated with an accelerating voltage of 300kV (magnification ~58,000X). The micrographs were digitized with a step size of 7 μm, resulting in a pixel size of 1.20Å on the object scale, and organized into 92 defocus groups with average defoci ranging from 1.20 to 4.52 μm. A total of ~130,000 particles were used for the reconstruction of the final map. The resolution (6.7Å) was estimated for the full data set by extrapolation from multiple resolution tests with increasing numbers of particles. Analysis of the map low-pass filtered to this resolution confirms the presence of details comparable to a similarly filtered X-ray map of the ribosome.

Compared to the previously published 10.25-Å resolution cryo-EM map of the same specimen, the new map represents a significant advance. Of particular interest in this complex is the binding interaction between the ribosome and the aa-tRNA•EF-Tu•GDP•kir ternary complex. Analysis of the map reveals local

conformational changes in the dynamic regions (i.e., effector regions) of the factor, and thus provides new insights into the mechanism of GTP hydrolysis during tRNA selection.

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277-Pos A-Site Ligand Mediates the L1 Stalk Dynamics of the Ribosome

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Board B110

Conformational dynamics of the ribosome are critical to the mechanism of translation . Recent accounts indicate that these dynamics are linked to the motions of transfer RNA (tRNA) on the ribosome . One dynamic region of the ribosome involved in tRNA translocation and release is the L1 stalk, which is located in the E site of the large subunit, and is believed to transition between opened and closed conformations . Here we present the first direct observation of tRNA motions with respect to the L1 stalk, before and after elongation factor G (EF-G)-mediated translocation using single-molecule fluorescence resonance energy transfer. We investigate the dependency of these dynamics on the nature of the A-site ligand, and their role in the mechanism of tRNA translocation.

278-Pos Cryo-EM Studies Of A Novel Ribosome Dimer From *E. Coli.*

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While most proteins in the ABC Transporter superfamily function in transmembrane transport, a substantial fraction are not associated with transmembrane domains and presumably function in other molecular processes. The best characterized of these is the fungal specific translation factor EF3, but the biochemical function has not been described for most of the others. We have shown that one such *E. coli* protein functions as a message-specific translational regulatory factor. A trans-dominant mutant of this protein blocks translation *in vitro* and *in vivo* and pulls down entire ribosomes. The bound complex consists at least in part of dimers of 70S ribosomes. While a ribosome dimer is a known storage complex formed during stationary phase in *E. coli*, formation of this previously characterized complex is dependent on proteins called Hpf and Rmf (Ueta et al., 2005; Wada et al., 1990), while the dimer complex formed by the ABC translation factor is not. We have been characterizing this novel ribosome dimer using a variety of techniques including cryo-EM.

279-Pos A High Resolution Cryo-EM Structure of T. Thermophilus Rigosomes

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Protein biosynthesis is one of the most important steps of gene expression and conducted by ribosomes. After the initiation of translation the ribosomes need elongation factors to further extend the nascent polypeptide chain. In bacteria the canonical elongation factor EF-Tu delivers the required amino acids (bound to tRNA) in complex with GTP (the EF-Tu•aa-tRNA•GTP ternary complex). We present here a cryo-EM map of the ternary complex bound to the T. thermophilus 70S ribosome. Image processing techniques allowed us to divide the initial data set into five different subsets corresponding to conformational changes present in the ribosomal complex. We used the data subset with the largest number of particle projections for further refinement to yield an improved electron density map (6.4 Å resolution using the 0.5 FSC criterion) that allows us to analyze the 3D arrangement of the three ribosomal ligands EF-Tu•aa-tRNA•GTP ternary complex, P-site tRNA and E-site tRNA. Because the crystal structure of the ribosome from T. thermophilus was recently solved at 2.7 Å resolution (1), a direct comparison of our cryo-EM map to the crystal structure is possible. This comparison leads to a complete interpretation of the ribosomal decoding complex in molecular terms and reveals important molecular interactions.

References

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280-Pos Visualization of Spontaneous Ratchet Motion in the Ribosome

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During the elongation step of translation, after the peptidyl transfer reaction, the peptidyl A-site tRNA on the ribosome moves to the P site, and the deacylated P-site tRNA moves to the E site. This process, termed translocation, is catalyzed by elongation factor G (EF-G). The detailed mechanism, at the atomic level, by which translocation occurs has yet to be elucidated. A recent single-molecule fluorescent resonance transfer (sm-FRET) study sug-

gested that, prior to completion of the translocation, the ribosome oscillates between three states characterized by three configurations of the tRNAs: the classical state (A/A and P/P), the hybrid state (A/P and P/E), and a previously unidentified hybrid state (A/A and P/E) in which the A and P site tRNAs have moved independently (Munro et al., 2007). Another study suggested that the spontaneous motions of the tRNAs are coupled with overall conformational changes of the ribosome (Ermolenko et al., 2007). Using cryo-electron microscopy, we collected over 200,000 projection images of ribosomal pre-translocational complexes carrying a single mutation on the P-loop (G2252C; see Munro et al., 2007). By applying classification to the projection images, we discovered two intrinsic conformations of the complex, one with the ratchet motion and the other without. This finding provides a structural confirmation for the idea that the ratchet motion is encoded in the ribosome architecture itself (Tama et al., 2003), that it can occur spontaneously, and that it provides the dynamics underlying mRNA-tRNA translocation (Frank et al., 2007).

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DNA, RNA Structure & Conformation

281-Pos High-Throughput Fluorescence Method to Determine Thermodynamic Parameters of Nucleic Acids

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Board B114

The nearest-neighbor model provides accurate predictions of stability for nucleic acid duplexes. These predictions are important for design of many biological applications. The model parameters have been traditionally obtained by ultraviolet spectroscopic and calorimetric methods. Since these techniques are low-throughput, thermodynamic parameters have yet to be determined for many significant chemical modifications (e.g., locked nucleic acids, phosphorothioate DNAs, 2′-O-methyl RNAs). Therefore, the effects of these modifications on duplex stability cannot be accurately predicted. We employed dyes and quenchers attached to nucleic acids, so that duplex melting transitions were accompanied by changes in fluorescence. Melting profiles were measured simultaneously for hundreds of duplex DNA samples using modified real-time PCR systems. Various designs of oligonucleotides, dyes and quenchers (FAM, HEX, TET, ROX, Cy3, Cy5, MAX, Rhodamine green, Texas Red, TAMRA, Tye563, Tye665, Alexa Fluor 488, Alexa Fluor 546, Black Hole and Iowa Black Quenchers) were studied. Their ability to monitor duplex denaturation and provide accurate values of enthalpies, entropies and free energies was characterized. The fluorescence method was then used to determine the thermodynamic parameters for chimeric LNA-DNA duplex oligomers.

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282-Pos Electromechanical switching of aptamers

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Board B115

Electrode-immobilized, fluorescently labeled DNA aptamers for thrombin can be stimulated electromechanically using alternating electrical fields. Their motion can be detected optically using energy transfer between fluorophore and the metallic electrode. In the absence of protein ligands, the motion of the aptamers follows the charging response of the electrical double-layer, leading to relaxation-type behavior at kHz frequencies. If thrombin is bound to the aptamers, a second relaxation process appears at much lower frequencies. The motion of the aptamer-protein complex seems to be strongly damped, which may be attributed to hydrodynamic effects or the interaction with neighboring aptamer strands or aptamer-protein complexes. The slow relaxation process can be utilized for sensitive detection of thrombin.

283-Pos Biophysical Study of Native and Locked Nucleic Acids

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Board B116

Locked nucleic acids (LNA) are bicyclic ribonucleotide analogs that show enhanced affinity and specificity against nucleic acid targets. To improve understanding of these effects, the dependence of thermodynamic stability on solution composition was studied for a series of DNA-DNA, RNA-RNA, RNA-DNA and LNA-DNA chimeric duplexes using UV spectroscopic and calorimetric melting experiments. We found that LNA nucleotides do not change the amount of sodium counterions released when duplexes denature. Therefore, the model that predicts effects of sodium ions on stability of native DNA duplex oligomers is also accurate for LNA-DNA chimeric duplexes. Osmotic stressing technique demonstrated that LNA modifications decrease the amount of water molecules released upon duplex melting substantially.

284-Pos On the Importance of the N7 of Guanine for the Formation of DNA Frayed Wires

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Board B117

Oligodeoxyribonucleotides comprised of long, consecutive, terminal guanines, ($d(N_xG_y)$ or $d(G_yN_x)$, $x \geq 5$, $y \geq 12$), self-aggregate to form a polydisperse set of multi-stranded structures termed DNA frayed wires (FW). These structures exhibit extreme thermostability and resistance to chemical denaturants and are held together through