Dynamics of Peptidyl transferase center in the single ribosomes.

Guangtao Song[1], Mediha E Altuntop[1], Yuhong Wang[1]
Univesity of Houston, Department of Biology and Biochemistry, Houston, TX 77096

Introduction: The peptidyl transferase reaction of ribosome catalyzes a central process for the biological world: the generation of proteins. Based on structural information in this region, the ribosome is proposed to be a ribozyme which imples the origin of life from a "RNA world".

However, there are several problems regarding the stringency of this "ribozyme model". (a) more recently structural studies show that two proteins, L27 and L16, are very close in the center of the peptidy trasferase center; (b) none of the rRNA nucleotide has been identified that carries out the catalytic task, and ribosmal RNAs without any protein cannot catalyze the peptide bond formation. (c) no direct biochemical data are available regarding of the enzyme mechanism due to the rate-limiting step of tRNA binding prior of peptidyl transferase reaction in the ensemble.

Using our single molecule method, we will be able to study the biologically relevant catalytical mechanism of this important enzyme during the *fast, continuing* cycles of peptide elongation and test the stringnecy of the "ribozyme model".

In our approach, *in vitro* protein synthesis system based on bacterial ribosome is utilized on the glass surface for single molecule detection. The ribosome protein L27 labeled with fluorescence groups interacts with fluorescently-labeled tRNAs via FRET (Fluorescence resonance energy transfer) process. The ribosome thus labeled specifically at L27 with Cy5 is obtained and shows the same activity as WT MRE600 ribosome in the Poly(Phe) assay. The phenylalanine tRNA is labeled with Cy3 at either the ThioU (8) position (for E coli Phe-tRNA) or the Dihydro-U (16/17) position (for yeast Phe-tRNA). The ribosome pre-complex /post-complex with Cy3-labeled tRNA at the A-site/P-site generate FRET of 0.4 and 0.6 efficiencies respectively with the Cy5-labled L27 protein. We have observed the fluctuation of these FRET species and measured the kinetics and thermodynamics data. These results provide quatitative information regarding of the ribosome dynamics in this region. Our results show that ribosome dynamics are hierarchically arranged and L27 maybe the key group to trigger the global conformational change that controls the ribosome activity.