

Dynamics of translation using single molecule spectroscopy

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We have explored the underlying dynamics of translation using single-molecule methods. These experiments have harnessed both single-molecule fluorescence and fluorescence energy transfer to determine dynamic changes in both composition and conformation of the ribosome during translation. Recently, we have explored the ligand dynamics during translation initiation and elongation using novel detection strategies, including nanostructures called zero mode waveguides (ZMWs). These experiments allow us to track translation dynamics at biological concentrations (μM) of substrate tRNAs and factors, and have subsequently revealed the timing of events in initiation, and elongation and mechanism of inhibition of antibiotics. We have extended these investigations to eukaryotic translation. These results move towards true real-time observation of protein synthesis.

Monday, February 7, 2011 at 10.30 p.m.

Seminar Room, Health Centre

University of Mumbai, Vidyanagari Campus, Kalina

Structural studies of the HIV reverse transcription initiation complex

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RNA structure guides key functions in HIV infection and replication. The viral genome is RNA, and RNA-RNA and RNA-protein interactions pervade the viral life cycle. The first step in viral replication after entry into an infected cell is reverse transcription, which is catalyzed by a viral enzyme, reverse transcriptase (RT). RT initiates from a specific RNA assembly formed from a host tRNA^{Lys3} and viral genomic RNA; initiation is rate limiting for reverse transcription. We have investigated the structure of the 50 kDa RNA initiation complex between HIV genomic RNA and human tRNA^{Lys3}. We show that both RNAs undergo large-scale conformational changes upon complex formation. Using heteronuclear NMR methods, we have determined the secondary structure of the RNA-RNA complex. Our results show that formation of the 18bp primer helix with the 3' end of tRNA^{Lys3} drives large conformational rearrangements of the tRNA at the 5' end, while maintaining the anticodon loop for potential loop-loop interactions. We will describe the NMR methods and approaches we use to determine RNA conformation, and present preliminary crystallographic data on the complex with reverse transcriptase. These results explain the importance of RNA structure in controlling reverse transcription rates.

Monday, February 7, 2011 at 1200 hrs

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