**Tata Institute of Fundamental Research**
Survey No. 36/P, Gopanpally Village, Serilingampally, Ranga Reddy Dist., Hyderabad - 500107

# Seminar

## RNA-based regulation – Insights from NMR

## Harald Schwalbe

### Goethe University Frankfurt, Germany

Riboswitches are cis-acting gene-regulatory RNA elements that can function at the level of transcription, translation and RNA cleavage. We have investigated such riboswitch RNAs by biophysical techniques, in particular NMR spectroscopy.

The commonly accepted molecular mechanism for riboswitch function proposes a ligand-dependent conformational switch between two mutually exclusive states. According to this mechanism, ligand binding to an aptamer domain induces an allosteric conformational switch of an expression platform, leading to activation or repression of ligand-related gene expression. However, many riboswitch properties cannot be explained by a pure two-state mechanism. We could show that the regulation mechanism of the adenine-sensing riboswitch, encoded by the add gene on chromosome II of the human Gram-negative pathogenic bacterium Vibrio vulnificus, is notably different from a two-state switch mechanism in that it involves three distinct stable conformations. We characterized the temperature and Mg (2+) dependence of the population ratios of the three conformations and the kinetics of their interconversion at nucleotide resolution. The observed temperature dependence of a pre-equilibrium involving two structurally distinct ligand-free conformations of the add riboswitch conferred efficient regulation over a physiologically relevant temperature range. Such robust switching is a key requirement for gene regulation in bacteria that have to adapt to environments with varying temperatures. The translational adenine-sensing riboswitch represents the first example, to our knowledge, of a temperature-compensated regulatory RNA element (Reining et al., 2013).

Regulation of transcriptional riboswitches located in the 5'-untranslated regions of messenger RNA requires the temporal synchronization of RNA synthesis and ligand binding-dependent conformational refolding. Ligand binding to the aptamer domain of the riboswitch induces premature termination of the mRNA synthesis of ligand-associated genes due to the coupled formation of 3'-structural elements acting as terminators. We could show that for the guanine-sensing *xpt-pbuX* riboswitch from *Bacillus subtilis*, the conformation of the full-length transcripts is static: it exclusively populates the functional off-state but cannot switch to the on-state, regardless of the presence or absence of ligand. We show that only the combined matching of transcription rates and ligand binding enables transcription intermediates to undergo ligand-dependent conformational refolding.

***Tuesday, Feb 20th 2018***

***11:30 AM (Tea/Coffee at 11:00 AM)***

***Auditorium, TIFR-H***