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

Seminar

Structure and dynamics in gene regulation

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We use NMR spectroscopy to study the structure and dynamics of protein-protein and protein-DNA complexes involved in DNA transcription and DNA repair. Examples are: (i) the E.coli Lac repressor, (ii) the human XPF-ERCC1 DNA repair complex, and (iii) the Rad6/Rad18 ubiquitination complex.

In the E. coli lac operon the Lac repressor binds to the lac operator and controls expression of the lac genes. Structures of Lac repressor, its DNA binding domains (headpiece) and complexes thereof with DNA have been extensively studied by X-ray and NMR methods and a wealth of biochemical data exists for this system. More recently we studied a dimeric lac repressor, and analysed the NMR spectra of the protein in the Free State, the inducer bound and in the 90 kD complex with a lac operator with and without inducer bound. Our results emphasize the role of dynamics in the allosteric coupling of inducer binding and operator affinity.

Nucleotide excision repair (NER) is the major DNA repair pathway for the removal of UV-induced photoproducts and bulky adducts from DNA. The complex of ERCC1-XPF is responsible for incision of the damaged strand at the 5' side of the damage. We determined the structure of the complex of the C-terminal domains of ERCC1 and XPF that are responsible for the ERCC1/XPF interaction and that mediate DNA binding. Both domains consist of a double helix-hairpin-helix motif $(HhH)_2$, and are related by a pseudo two-fold symmetry axis. Structural differences are seen between ERCC1 and XPF that correlate with DNA binding for ERCC1. We propose a model for the targeting of XPF nuclease to the damage site via ERCC1 mediated interactions which may give ERCC1 a regulatory role in NER.

The human E2 Rad6 and the RING E3 ligase Rad18 are involved in the monoubiquitination of PCNA in response to DNA damage and play a crucial role in translesion DNA synthesis. Using a combination of NMR and crystallography we analysed the structures and the complex protein interaction network of Rad6, of the Rad18 RING and the second R6 binding domains, and of non-covalently and covalently attached ubiquitin. On the basis of these studies, we present a model for the mechanism of the monoubiquitination of PCNA by the tandem Rad6/Rad18.

Several complexes were modelled using our docking procedure Haddock. In many cases, the NMR studies were complimented with biophysical data and results from complimentary structural biology techniques. The different examples demonstrate the strength and flexibility of NMR for studying the structure and dynamics of proteins and complexes involved in cellular regulation.

Friday, Feb 14th 2019

4:00 PM (Tea/Coffee at 3:30 PM)

Auditorium, TIFR-H