Tata Institute of Fundamental Research
Survey No. 36/P, Gopanpally Village, Serilingampally, Ranga Reddy Dist., Hyderabad – 500 046

Seminar

Microscopy and image analysis based enhancements for investigating DNA damage responses in cells

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In a eukaryotic cell, genetic information is stored in DNA which is constantly subjected to change from various internal and external factors. Cells have evolved various mechanisms that detect and reverse these changes, maintaining information fidelity. These repair mechanisms are broadly classified as DNA damage responses (DDR), which are crucial for the proper functioning of the cell. In this thesis, DDR has been investigated spatio-temporally in cells with new modalities of microscopic imaging and analysis. Fluorescence anisotropy imaging has been combined with laser micro-irradiation to study chromatin compaction dynamics upon localised laser-induced damage. Localization and dynamics of repair factors and their correspondence to underlying chromatin structures post-damage were investigated. It was observed that chromatin compacts globally upon local damage, with phosphorylated forms of ATM, and PCNA forming possibly phase-separated nodes in regions of less compact chromatin, which also appeared to be regions of DNA synthesis. Further, in these experiments, sample size per experiment was found to be fundamentally limiting, and new software was developed to generically improve throughput in imaging assays. With these new tools of acquisition and analysis, the statistics of microscopic investigations in a regular widefield setup were vastly improved. In fixed-cell immunofluorescence assays, data were obtained for over tens of thousands of cells, and rare subpopulations could be identified. Cell-cycle linked post-damage events in living cells were captured without the use of cell-cycle blocks as a result of increased throughput in timelapse microscopy. Together this thesis uses fluorescence anisotropy-based imaging to study chromatin compaction in the context of DDR, and also further describes the development of new tools to improve the throughput of microscopy assays.

Wednesday, Dec 28th 2022

10:00 AM (Tea/Coffee at 9:45 AM)

Auditorium, TIFR-H