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

Internal Webinar

Deciphering the proteomic profile of active vs dormant origins of replication in the mammalian system

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Replication of the human genome, which spans more than 3 billion base pairs, is a highly demanding process. To complete this task within a single cell cycle, mammalian cells license tens of thousands of potential replication origins. However, only a small subset is activated during an unperturbed S phase, while the remaining stay dormant. These dormant origins serve as a backup pool, becoming activated under replication stress to ensure faithful genome duplication. The selective firing of origins provides both efficiency and robustness, yet raises a central question: what mechanisms determine which origins fire and which remain dormant?

The regulatory mechanisms, particularly at the proteomic level, that prevent the simultaneous activation of all origins and maintain the dormancy of inactive origins during S phase remain poorly understood. It is not yet clear how the proteome at active origins differs from that at dormant origins, or what regulatory implications this distinction carries. While extensive studies have examined fired origins and their protein interactions, the proteome of dormant origins in mammalian systems remains largely unexplored.

To address these questions, we are using mammalian cells as a model to investigate the proteomic profiles of active and dormant origins. We will employ CRISPR-Cas9–mediated endogenous knock-in of MCM subunits with protein tags such as EGFP, HaloTag, and FLAG to enable efficient visualization and purification of MCM proteins and their interacting partners at both dormant and active origins. This will be combined with click-chemistry–mediated pulldown of chromatin in S-phase cells. Finally, mass spectrometry–based proteomic analysis will be conducted to perform comparative and functional studies of the protein pools associated with active and dormant origins. Through this approach, we aim to reveal the regulatory protein networks that govern origin firing versus dormancy, providing new insights into the control of DNA replication in mammalian cells.

Thursday, Sep 18th 2025

16:00 Hrs