SESSION 1C

MOLECULAR CONTROL OF THE CELL

Session Moderator: Hannele Ruohola-Baker, Biochemistry
MGH 171
12:30 PM to 2:15 PM
* Note: Titles in order of presentation.

The Role of the Replication Fork Barrier in rDNA Instability

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Genes encoding the RNA portion of the ribosome (rDNA) are present in essentially all eukaryotic genomes as tandem repeated arrays. In humans, rDNA copy number is highly variable and an undervalued potential source of genetic disease. Changes in rDNA copy number can occur through DNA breakage and repair as well as through errors in DNA replication. High transcriptional activity at the rDNA locus poses challenges for replication; all tested eukaryotes have evolved replication fork barriers (RFBs), ensuring that replication machinery does not collide with transcribing RNA polymerases. In yeast, the RFB is a specific sequence to which the protein Fob1 binds, blocking replication forks that converge with transcription. Mutants lacking Fob1 have greatly reduced variation in rDNA copy number. There are currently two models to explain how Fob1 binding to the RFB produces rDNA copy number instability. One model suggests that binding of Fob1 actively recruits DNA break and repair machinery which induces recombination between rDNA repeats. Another model proposes that the stalled replication fork at the RFB is inherently fragile, increasing the likelihood of breakage. To distinguish between these two models, I am generating yeast strains where Fob1 binds to the RFB but does not arrest forks. Using CRISPR/Cas9 gene editing technology, I am reversing the direction of the RFB in each of the 150 rDNA repeats in yeast to prevent replication fork stalling. By confirming the absence of replication fork stalling and determining whether rDNA instability has also been reduced, I can distinguish whether Fob1 binding to the RFB in the absence of fork blocking contributes to rDNA copy number changes. Clarifying involvement of the RFB in rDNA copy number changes will additionally provide insights into the connections between transcriptional activity, replication fork stalling and genome instability.