Suppression of mitochondrial transcription initiation complexes changes the balance of replication intermediates of mitochondrial DNA and reduces 7S DNA in cultured human cells

曲, 建華

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Human mitochondrial DNA (mtDNA) is a closed circular molecule of approximately 16.6 kb. A series of studies proposed that mammalian mitochondria operate two replication modes. One is a novel DNA replication mechanism, designated as RITOLS. In this replication mode, the major initiation position of H strand DNA synthesis is Ori-H (origin of mtDNA heavy strand synthesis). The other mode is coupled leading and lagging DNA strand synthesis in which initiation starts at a broad zone in mtDNA. According to the studies of replicating mammalian mtDNA, the L-strand transcription promoter is located upstream of Ori-H suggested a role for this promoter in initiation of mtDNA replication. Here, I investigated the relationship between mitochondrial transcription initiation and mtDNA replication initiation by analyzing the effect of knockdown of mitochondrial transcription factor B2, TFB2M and mitochondrial RNA polymerase, POLRMT, components of the transcription initiation complexes in cultured human cells. Suppression of transcription initiation complex induced by TFB2M and POLRMT knockdown causes a reduction in replication intermediates of mtDNA RITOLS replication mode. Beyond that, replication intermediates of coupled leading and lagging strand DNA replication seemed to be less affected. The findings support the view that the RITOLS replication mode involves transcription from the light strand promoter, and significantly suggest that the initiation of the coupled leading and lagging strand DNA replication is independent from the transcription. Based on the findings, I demonstrate that in “living mammalian cells”, LSP-dependent transcription is confirmed to be involved in the initiation of mtDNA replication.