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MOLECULAR INSIGHTS INTO MITOCHONDRIAL DNA REPLICATION

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ABSTRACT

Mitochondria are organelles found in eukaryotic cells. These organelles produce most of the adenosine triphosphate that cells use as a source of energy. Mitochondria contain their own genomic material, a circular DNA genome (mtDNA) that encodes subunits of the respiratory chain complexes and RNA components needed for mitochondrial translation. Many aspects of mtDNA replication are still not understood and in this thesis we address some of the molecular mechanisms of this process in mammalian cells.

DNA synthesis cannot be initiated de novo, but requires a short RNA primer as a starting point. We here demonstrate that the mitochondrial RNA polymerase (POLRMT) is the primase required for initiation of DNA synthesis from the origin of light strand DNA replication (OriL) in human mtDNA. Using purified POLRMT and the core factors of the mitochondrial replisome, we faithfully reconstitute OriL-dependent initiation of replication in vitro. During origin activation, OriL is exposed in its single-stranded conformation and adopts a stem-loop structure. POLRMT initiates primer synthesis from a poly-dT stretch in the single-stranded loop region and after about 25 nt, POLRMT is replaced by the mitochondrial DNA polymerase γ (POLγ) and DNA synthesis is initiated. Our findings also suggest that the mitochondrial single-stranded DNA binding protein directs origin-specific initiation by efficiently blocking unspecific initiation events in other regions of the mtDNA genome.

To analyze the requirements of OriL in vivo, we have used saturation mutagenesis in the mouse combined with in vitro biochemistry and demonstrated that OriL is essential for mtDNA maintenance. OriL requires a stable stem-loop structure and a pyrimidine-rich sequence in the template strand for proper origin function. The OriL mechanism appears to be conserved, since bioinformatics analyses demonstrated the presence of OriL in the mtDNA of most vertebrates including birds. Our findings suggest that mtDNA replication may be performed by a common mechanism in all vertebrates and lend support to the strand-displacement model for mtDNA replication.

A molecular understanding of the mitochondrial DNA replication machinery is also of medical importance. Today, more than 160 mutations in the gene encoding the catalytic subunit of POLγ (POLγA) have been associated with human disease. One example is the Y955C mutation, which causes autosomal dominant progressive external ophthalmoplegia, a disorder characterized by the accumulation of multiple mtDNA deletions. The Y955C mutation decreases POLγ processivity due to a decreased binding affinity for the incoming deoxyribonucleoside triphosphate. However, it is not clear why this biochemical defect leads to a dominant disease. We have used the reconstituted mammalian mtDNA replisome and studied functional consequences of the dominant Y955C mutation. Our study revealed that the POLγA:Y955C enzyme is prone to stalling at dATP insertion sites and instead enters a polymerase/exonuclease idling mode. The mutant POLγA:Y955C competes with wild-type POLγA for access to the primer template. However, once assembled in the replisome, the wild-type enzyme is no longer affected. Our data therefore provide a mechanism for the mtDNA replication phenotypes seen in patients harboring the Y955C mutation.

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