

ABSTRACT

ROLES OF THE MITOCHONDRIAL SINGLE-STRANDED DNA-BINDING PROTEIN AT THE MITOCHONDRIAL DNA REPLICATION FORK

By

Marcos Túlio Oliveira

The mitochondrion is one of the most important and versatile eukaryotic organelles, responsible for the bulk of energy production in the cell, and involved in apoptosis, signaling, cellular differentiation, and control of cell cycle and cell growth. It possesses its own genome, the mitochondrial DNA (mtDNA), which encodes polypeptides that are essential subunits of the complexes that form the energy-producing respiratory chain. mtDNA replication is thus an important process that maintains proper mitochondrial function, accomplished by the coordinated action of three main protein components that work directly at the mtDNA replication fork: DNA polymerase γ (pol γ), which catalyzes DNA synthesis per se; mtDNA helicase (also known as Twinkle), which unwinds double-stranded DNA to provide a single-stranded DNA (ssDNA) substrate for pol γ ; and mitochondrial single-stranded DNA-binding protein (mtSSB), which binds ssDNA to protect it against damage and to coordinate the functions of pol γ and mtDNA helicase. The general aim of my thesis work was to examine the roles of mtSSB at the mtDNA replication fork by investigating the biochemical and physiological performance of a group of mtSSB variants bearing alanine substitutions or deletions of amino acid residues conserved across animal species. We purified 9 recombinant human mtSSB (HsmtSSB) proteins, which maintained their homotetrameric state and bound ssDNA with only slightly different affinities. However, they exhibited very distinct capacities to stimulate the DNA polymerase activity of human pol γ (Hspol γ) and the DNA unwinding activity of human mtDNA helicase

(HsmtDNA helicase) *in vitro*. Whereas the variants HsmtSSB Δ N (Δ 1-9), Δ C (Δ 126-132) and Δ N Δ C (Δ 1-9, Δ 126-132) stimulated Hspol γ ~2-fold higher than HsmtSSBwt (wild type), the variants HsmtSSBloop23 (Δ 51-59), α 1 (Y83A/Q84A) and loop45-1 (Y100A/G101A/E102A) exhibited an ~40% reduction as compared to HsmtSSBwt. We developed a molecular model of mtSSB-pol γ interaction that explains these and other biochemical data published previously, and that suggests how a group of Hspol γ mutations associated with various human diseases may disturb such interactions. Interestingly, the variants HsmtSSBloop12 (E33A/G34A/K35A) and loop45-2 (K106A/N107A/N108A), which did not exhibit altered capacity to stimulate Hspol γ , were indeed defective in stimulating HsmtDNA helicase, presumably by failing to interact with its C-terminal tail. We also evaluated mtSSB in *Drosophila* S2 cells by knocking down the endogenous protein (DmmtSSB) and expressing variants of DmmtSSB equivalent to those of HsmtSSB. Endogenous DmmtSSB knockdown and overexpression of DmmtSSB variants caused reduction of mtDNA copy number under conditions of mitochondrial homeostasis, and impeded mtDNA repletion during recovery from treatment with ethidium bromide, when mtDNA replication is stimulated *in vivo*. Preliminary analysis of mtDNA replication intermediates from cells overexpressing DmmtSSB variants indicated that the defects in mtDNA replication are associated with the binding sites of the transcription termination factor DmTTF. Our findings suggest that mtSSB uses a repertoire of structural elements to interact functionally with pol γ and mtDNA helicase, to guarantee proper mtDNA replication in animal cells. Understanding the mechanisms of mtDNA replication via further biochemical, physiological and structural studies will provide valuable insights into the processes in which the mitochondrion is the key regulator in eukaryotic cells