DNA synthesis determines the binding mode of the human mitochondrial single-stranded DNA-binding protein

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J. Morin[I], J. Jarillo[II], F. Cerron[III], E. Beltran-Heredia[II], G. Ciesielski[IV], L.S. Kaguni[IV], F.J. Cao[II], **B. Ibarra**[III]

[I]BIOTEC, Dresde, Germany, [II]Universidad Complutense Madrid, Madrid, Spain, [III]IMDEA Nanociencia, Madrid, Spain, [IV]Michigan State University, Lansing, United States of America

Single-stranded DNA-binding proteins (SSBs) play a key role in genome maintenance, binding and organizing single-stranded DNA (ssDNA) intermediates. Multimeric SSBs, such as the human mitochondrial SSB (HmtSSB), present multiple sites to interact with ssDNA, which has been shown in vitro to enable them to bind a variable number of single-stranded nucleotides depending on the salt and protein concentration. It has long been suggested that different binding modes might be used selectively for different functions. To study this possibility, we used optical tweezers to determine and compare the structure and energetics of long, individual HmtSSB-DNA complexes assembled on preformed ssDNA, and on ssDNA generated gradually during 'in situ' DNA synthesis. We show that HmtSSB binds to preformed ssDNA in two major modes, depending on salt and protein concentration. However, when protein binding was coupled to strand- displacement DNA synthesis, only one of the two binding modes was observed under all experimental conditions. Our results reveal a key role for the gradual generation of ssDNA in modulating the binding mode of a multimeric SSB protein and consequently, in generating the appropriate nucleoprotein structure for DNA synthetic reactions required for genome maintenance.