The effects of a higher order DNA structure on mitochondrial recombinase reactions
ミトコンドリアの相同組換え酵素反応への DNA の高次構造の効果

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It is believed that the major form of mitochondrial DNA (mtDNA) is closed circular double-stranded (cc-ds). Cc-dsDNA isolated from living organisms has right-handed (negative) supercoils without exception. Since negative supercoils are relaxed by untwisting of double helix, various non-B form structures of dsDNA such as local melting, left-handed helix and cruciform are stabilized by negative supercoils. Homologous recombination is initiated by double-stranded breakage followed by the processing of the strand-termini to generate 3' single-stranded (ss) tails. A recombinase binds to the ss-tails and catalyzes the invasion of a tail into intact dsDNA to form a heteroduplex joint with the complementary sequence within the dsDNA ("homologous pairing"). As the results, a homologous three-stranded structure consisting of the heteroduplex joint and a loop of the replaced strand is formed ("D-loop"). Since the formation of D-loops associates with the melting of double-helix, D-loop is stabilized by negative supercoils. During homologous pairing, the switch from parental base pairs to those between the invading ssDNA and the complementary strand of the dsDNA requires untwisting of the parental double helix to bypass structural constraints. Actually, homologous pairing catalyzed by RecA is extensively accelerated[1]. Generally, homologous pairing in homologous recombination is catalyzed by a RecA/Rad51-family protein in bacteria and nuclei of eukaryotic cells. However, a yeast *Saccharomyces cerevisiae* mitochondrial recombinase, Mhr1, does not require ATP. Some proteins in addition to Mhr1 were found to catalyze D-loop formation with negatively supercoiled dsDNA in the absence of ATP. Since these ATP-independent proteins have potent annealing (dsDNA formation from complementary ssDNA) activity, it is a matter of debate whether the ATP-independent D-loop formation is homologous pairing between ssDNA and dsDNA, or annealing between ssDNA and a local melting region of negative supercoiled dsDNA. In addition, since mtDNA has no chromatin structures that relieve topological stress derived from negative supercoils, it is assumed that mtDNA is under topological stress. To obtain an insight into these matters, we examined the effects of the topological stress on Mhr1-catalyzed homologous pairing, and obtained results that suggest a novel mechanism of the homologous pairing catalyzed by Mhr1 and status of DNA in mitochondria.

References:
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