BIOMATERIALS

Forces in Biomolecular Systems SFB 863

DNA Secondary Structure Formation in Bacterial Gene Capture Systems at Single-Molecule Resolution

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Bacteria with multiple antibiotic resistances are a threat to human health. These resistances spread faster than could be expected from mutations alone. It was shown that bacteria exhibit a mechanism of exchanging and collecting genetic sections – coding for antibiotic resistances or other adaptive traits – between individuals or even across species boundaries.

This mechanism relies on genetic elements termed integrons. They allow the incorporation and expression of exogenous gene cassettes through a site-specific recombination process. The process involves an enzyme, integrase, which mediates recombination between a double stranded integron recombination site (attI site) and a single-stranded cassette recombination site (attC site). The attC site is supposably recognized by integrase through specificity to the secondary structure of the DNA hairpin formed by the single-stranded attC site.

This poses the question of how the DNA hairpin forms inside the living cell. It was shown in vivo that negative superhelicity promotes integron recombination, most likely through cruciform extrusion from double-stranded DNA.

Here, we use single-molecule FRET and magnetic tweezers to study the formation of the postulated DNA hairpin in the presence of various proteins. We present data on the competition between SSB and integrase and on the extrusion of E. coli attC sites from dsDNA and their interaction with integrase hinting at possible molecular mechanisms underlying the function of this system in bacteria.

Friday, November 22nd, 2013, 13:00

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