The effect of cytoskeletal crowding on the Mobility and Conformational Dynamics of Circular and Linear DNA

Rachel Dotterweich, Kathryn Regan, Shea Ricketts, Sylas Anderson, Rae M. Robertson-Anderson
University of San Diego

We characterize DNA dynamics when crowded by cytoskeletal protein networks

Within the cell, diffusion of DNA through the cytoskeleton is necessary for many important processes such as drug delivery and suppressing viral transfection. The cytoskeleton is comprised of semiflexible actin filaments and rigid microtubules. However, how each filament affects the diffusion of DNA through the cytoskeleton remains unknown. Further, while DNA naturally exists in both linear and circular forms, how the DNA topology impacts its diffusion through the cytoskeleton is also not known.

We track single linear and circular DNA molecules diffusing through networks of actin and microtubules

DNA is added to 11.6 µM actin monomers and/or tubulin dimers. Polymerization of actin and/or microtubules is achieved by adding 10 mM ATP and/or GTP and incubating at 37°C for 30 mins.

115 kbp linear or circular DNA is labeled with fluorescent dye YOYO-1 at a 1:5 dye:basepair ratio.

Single DNA molecules are imaged and tracked with an epifluorescence microscope with a GOX objective. We record 30 sec videos at 10 frames per second. >50 molecules are tracked for each condition.

The center-of-mass MSDs in x and y directions determine the diffusion coefficients (D). The lengths of the major and minor axes of the DNA measure the effective coil size (R) and the length-scale (λ) and rate of conformational fluctuations (β).

Interactions between actin and microtubules are needed to appreciably slow the fluctuation rates of both linear and circular DNA

We measure the effect that crowding by cytoskeletal networks has on the diffusion of linear and circular DNA

Cytoskeletal networks slow the diffusion of circular DNA more than linear DNA

Cytoskeleton crowding compacts both linear and circular DNA

Cytoskeleton crowding slows the rates that DNA fluctuate between conformational states but increases the range of accessible states

Reduced DNA Coil Size

Microtubules have minimal effect on DNA conformation
Composites compact DNA more than single component networks

Reduced Fluctuation Rates

Actin or microtubules alone have little effect on circular DNA fluctuation rates
Faster circular DNA fluctuations are coupled to slower diffusion

Reduced Steady-State Fluctuation Lengths

Crowding increases the range of conformational states accessed

References