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Real-Time Analysis of Endogenous Nuclear NADH in Differentiating Cells using the Spectral Phasor Approach

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NADH is an endogenous autofluorescent regulatory metabolite detected in the nuclear regions of live cells which when analysed for the bound and free form can aid in determining a cell's metabolic status and molecular activity. Detecting the spectral differences of free and bound NADH in live cells is currently limited due to the very small differences in emission. The Spectral Phasor technique enables not only examination of small shifts in spectral emissions but also provides the spatial location of spectrally different components in live cells without any prior knowledge of the species. The phasor representation enables direct comparison of either optical sections (i.e. different focal planes) of one cell or multiple cells for global analysis.

Here we describe the use of Spectral Phasors to spatially map NADH's spectral emission in the nucleus of live cells under normal culture conditions and those stimulated into the early stages of differentiation. A comparison of undifferentiated cells and those stimulated to differentiate demonstrate differing spatial distributions of emission spectra associated with NADH. Undifferentiated cells displayed shorter emissions cantered in the nucleus, while longer wavelengths were localised around the perinuclear boarder. Cells stimulated into the early stages of differentiation displayed a redirection of the shorter emissions to regional clustering predominately in one area close to the nuclear/cytoplasmic boarder, while the longer wavelengths were localised throughout the remainder of the nucleus. Here we show the application of the Spectral Phasor technique to identify discrete wavelength shifts associated with endogenous NADH autofluorescence in the nucleus, and observed changes in their spatial distribution in live cells during the early stages of differentiation. Work in part supported by NIH P41-GM103540 and NIH P50-GM076516.

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Plasmid-Encoded Noncoding RNA Regulates Chromosomal Gene Expression

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It is known that introducing a plasmid into bacteria leads to metabolic burden and host gene expression alteration, and that introduction of ColE1-type plasmid into bacteria also changes global metabolic pathways. However, the underlying mechanisms coordinating these changes are still unclear. Since ColE1-type plasmids are widely used in many biological fields, understanding the mechanism underlying the effects of the ColE1 plasmid on host gene expression is critical. In our study, we focus on the plasmid-encoded noncoding RNA that normally negatively regulates plasmid copy number. We found that ectopic expression of plasmid-encoded noncoding RNA on control plasmid can reverse the host gene expression changed by introduction of the control plasmid itself. Further, using in vitro gel shift assay we found that the plasmid-encoded noncoding RNA can bind to host factor Hfq. Furthermore, deletion of Hfq decreases the expression level of plasmid-encoded noncoding RNA. Together, we propose that ectopically expressed, plasmid-encoded noncoding RNA may compete with host RNA species for Hfq binding, which further disrupts host gene expression normally responsive to introduction of plasmid itself.

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TrmBL2 Protein from Thermococcus Kodakarensis Competes with Histones for DNA Binding and Forms Filamentous Nucleoprotein Complexes that Affect DNA Structural State

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Architectural DNA proteins play important roles in the genome organization and maintenance of its functionality in living cells. During past years DNA binding properties of different architectural proteins from bacterial and eukaryotic cells were extensively investigated. However, physiological functions of some DNA-architectural proteins from archaeal cells still remain unclear. Recently, several abundant DNA-architectural proteins including histones, Alba, and TrmBL2 have been identified in model euryarchaeon T. kodakarensis. While histones and Alba proteins have been characterized in detail, the DNA-binding properties of TrmBL2 remain largely unexplored. Here, we report single-DNA studies showing that TrmBL2 binds to DNA with positive cooperativity, resulting in formation of rigid nucleoprotein filaments. Our results indicate that polymerization of such filaments on DNA reduces with increasing ionic strength of solution due to the electrostatic screening of TrmBL2 non-specific binding to DNA, which is nearly abolished at $>$ 300 mM KCl. Yet, patches of DNA covered by TrmBL2 can be seen on AFM images even at large salt concentrations, suggesting presence of high affinity TrmBL2-filament nucleation DNA sequences. Further DNA footprint analysis revealed that TrmBL2 preferentially interacts with G/C-rich sequences, similar to archaeal histones, thus, suggesting existence of TrmBL2-histone binding competition to overlapping DNA segments. This observation is supported by competitive binding assay, showing that TrmBL2 and archaeal histones indeed mutually occlude each other for DNA binding. Finally, DNA twisting experiments showed that TrmBL2-filaments weaken formation of positively supercoiled plectonemes during DNA winding and promote DNA transition into negatively supercoiled Z/L-state during unwinding. Taken together, these results advance our understanding of TrmBL2 DNA-binding properties and provide important insights into its potential functions in nucleoid organization and gene regulation in hyperthermophilic euryarchaea cells.

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Free Energy Profiles for Nucleosomal DNA Unwrapping

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Eukaryotic genome is compactly stored into a tiny nucleus of cell in a form of protein-DNA complex. This protein-DNA complex is called as nucleosome which is composed of a histone octamer formed by two copies each of the four core histones H3, H4, H2A and H2B and about 150bp of DNA wrapping almost twice around the octamer. This compact form, however, has to be unwrapped in transcription, DNA duplication and DNA repair processes. To study the detailed molecular mechanism, we calculated free energy profiles for unwrapping nucleosomal DNA on H3-containg and CENP-A containing nucleosomes with adaptively biased MD and umbrella sampling. We estimated from the profiles that a cost for unwrapping the outer DNA is 0.1 to 0.4 kcal/mol/1bp, consistent with a value experimentally obtained. Compared with H3 nucleosome, the profile showed that CENP-A nucleosome was the most stable in a conformation where 15bp from each of ends were unwrapped. The detail analysis indicated that this is attributable to the difference in ability of forming hydrogen bond of αN helix of H3 and CENP-A. We unwrapped DNA further from the histone core up to 38bp from DNA ends. Relative distance matrix analysis showed that the distance between H2A-H2B and H3-H4 increased in a process of 0 to 17bp unwrapping. This distance decreased during a process of 17bp to 25bp unwrapping, indicating that the conformational relaxation occurred in the histone core.

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Studies of the Compaction Mechanisms of DNA-Binding Proteins using Horizontal Magnetic Tweezers

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In order to study the functional mechanisms of DNA compacting proteins, we have developed a novel transverse magnetic tweezers that can apply tension to single DNA molecules in the horizontal plane with forces ranging from ~0.01 to ~10 pico-Newtons. We use a λ- DNA attached to a 2.8 μm superparamagnetic bead on one end and 3 μm polystyrene bead on the other end. We describe the tweezers in detail and present data validating its performance. We show that using a relatively simple design complemented with image processing techniques for subpixel detection, we can reliably measure changes in the DNA molecule's extension of less than 7 nm over a force range suitable for studying the binding of proteins. This is enabled by the horizontal geometry of the applied force allowing both beads to remain in the focus plane throughout an experiment. We also describe a protein microspray technique which we use to introduce proteins in the presence of an extended tether. Next, we present data from experiments probing the binding of histones to DNA and their force-induced unbinding. When the force is increased to beyond ~2 pN, we find the tether re-extends with characteristic jumps in extension of multiples of ~50 nm. These may signal the disruption of nucleosomes or non-canonical DNA-histone complexes. We conclude with a discussion of our experiments on the binding mechanism of the protein mIHF that plays an important role in the infection pathway of tuberculosis.