intensity of the Spinach-DFHBI RNA aptamer fluorogen complex hampers its utility in quantitative live-cell and high-resolution imaging applications. Here we report that illumination of the Spinach-fluorogen complex induces photoconversion and subsequently fluorogen dissociation, leading to fast fluorescence decay and fluorogen concentration-dependent recovery. We demonstrate a low-repetition illumination scheme that enables us to maximize the potential of the Spinach-DFHBI RNA imaging tag in living cells.

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Identification and Analysis of the Transcriptional Regulatory Networks Governing Mechanosensitive Channels in E. coli

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Mechanosensitive (MS) channels are membrane proteins which are gated by mechanical stress in the cell membrane. Channel opening in response to mechanical stresses makes it possible for molecules to pass through the membrane, allowing an organism to alleviate solute imbalances which place osmotic stress on the membrane and can cause cell lysis and death. Two of the seven known MS channels in E. coli, MscL and MscS, are individually capable of rescuing these bacteria from sudden changes in osmolarity (osmotic shock). If MscL and MscS are both sufficient for ensuring survival under osmotic shock conditions, what is the purpose of the other five MS channels? In the interest of exploring the physiological roles of the various MS channels, we hypothesize that understanding how they are regulated will shed light on their function. Specifically, we investigate the regulatory context of the MS genes by identifying the regulatory architecture and transcription factors responsible for controlling MS gene output. We use a method known as "Sort-Seq" to locate transcription factor binding sites with base pair resolution, and DNA affinity chromatography to determine transcription factor identity. Understanding how the MS genes are regulated will give us clues as to the specific stressors which they defend the cell against. This work provides a specific case study of a method which we think will have much broader reach in mapping out regulatory networks genome-wide.

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Cooperativity and Interaction Energy Threshold Effects in Recognition of the −10 Promoter Element by Bacterial RNA Polymerase

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RNA polymerase (RNAP) melts promoter DNA to form transcription-competent open promoter complex (RPo). Interaction of the RNAP σ subunit with non-template strand bases of a conserved −10 element (consensus sequence $T_{-12}A_{-11}T_{-10}A_{-9}A_{-8}T_{-7}$) is an important source of energy-driving localized promoter melting. Here, we used a fluorometric RNAP molecular beacon assay to investigate interdependencies of RNAP interactions with −10 element nucleotides. The data reveal a strong cooperation between RNAP interactions with individual −10 element non-template strand nucleotides and indicate that recognition of the −10 element bases occurs only when the overall RNAP −10 element binding acquires a free energy below a ca. −3 kcal/mol threshold. These results may be explained by that the individual interactions between RNAP and −10 element nucleotides cooperatively contribute into retaining a characteristic recognizable conformation of the −10 element nt-strand that was revealed in recent structural studies. The RNAP interaction with T/A_{-12} base pair was found to be strongly stimulated by RNAP interactions with other −10 element bases and with promoter spacer between the −10 and −35 promoter elements. The data also indicate that unmelted −10 promoter element can impair RNAP interactions with promoter DNA upstream of the −11 position. We suggest that cooperativity and threshold effects are important factors guiding the dynamics and selectivity of transcription initiation complex formation.

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Super-Resolution Fluorescence Microscopy of Transcription Sites in E. coli

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Bacterial processes have long been thought of to occur in the cytoplasm without further internal organization such as found for eukaryotes. Yet, recent studies increasingly reveal that many processes are spatially organized, also including transcription [1].
As diffraction-limited fluorescence microscopy is unable to resolve the precise distribution of active RNA polymerases we apply quantitative photoactivation-localization microscopy (PALM) using the monomeric and bright fluorophore PAmCherry1 [2, 3].
We show super-resolved mapping of the spatial organization of RNA polymerase in E. coli under different growth conditions, apply various concepts of quantitative analyzes of the localization microscopy data and discuss the limits of localization-based super-resolution techniques in assessing biomolecular structures with high spatiotemporal resolution.
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Characterization of Transcription Initiation Intermediates in Escherichia Coli RNA Polymerase by Fluorescence

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Transcription initiation involves a series of conformational changes that are triggered in both RNA polymerase (RNAP) and DNA after recognition of −35, −10 elements of promoter DNA by RNAP holoenzyme, consisting of catalytic core and a specificity factor, σ. RNA polymerase (RNAP) functions as a molecular isomerization machine, binding and bending the promoter DNA in the initial closed complex and using binding free energy to melt 13 bp of DNA in the active site cleft to form an initial unstable open promoter complex, which is converted to a final open complex (RPo) (Saecker et al, '11). Promoter melting is thought to commence upon flipping of −11A of the non-template strand out of the DNA helix (Schroeder et al, '09) but several questions remain open. After recruitment of RNAP to promoter DNA, what conformational changes occur in the complex to put the downstream DNA duplex in the cleft? When and how is the DNA bent and wrapped around RNAP? When does the −11A element rotate vis-à-vis wrapping? To answer these questions, we are characterizing early intermediates at the λPr promoter using equilibrium and kinetic bulk fluorescence measurements. Fluorescence Resonance Energy Transfer (FRET) experiments monitor the extent of wrapping whereas stopped-flow ''Beacon'' assays provide information about −11A base-flipping and opening of −10 region (Mekler et al, '11). FRET experiments show that promoter DNA is wrapped around RNAP in the advanced closed complex and that wrapping persists in open complexes. The wrapped open complex is found to be transcriptionally competent. Real-time stopped-flow kinetic experiments are in progress to determine the sequence of steps in which upstream and downstream duplex promoter DNA are bent toward and wrapped around RNAP to put the start site region in the cleft and open it.

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Activating the Prokaryotic *leu-500* Promoter by Transient, Dynamic DNA Supercoiling in Escherichia Coli

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DNA supercoiling is a fundamental property of chromosomal DNA in living cells and greatly affects the efficiency of many essential DNA transactions. For instance, the prokaryotic *leu-500* promoter, an inactive promoter in bacteria, and can be activated by supercoiling in the *topA* strains. Although DNA supercoiling around the promoter region was considered playing an essential role in the activation of the *leu-500* promoter, whether the global or the localized supercoiling carries out the activation is not clear. In this study, we developed two *in vivo* systems to study how transient, dynamic DNA supercoiling (TDDS) activates the *leu-500* promoter. The first system consists of *E. coli topA* strain *VS111(DE3)* and a linear plasmid. The *topA* strains provided an IPTG-inducible T7 RNA polymerase for TDDS and also does not have a functional topoisomerase I to remove the (-) supercoiled domain. In this case, the (-) supercoiled domain should exist for longer time. The linear plasmid cannot be globally supercoiled, which provided an excellent template to study TDDS