MAR17-2016-020460

Abstract for an Invited Paper
for the MAR17 Meeting of
the American Physical Society

Determination of in vivo regulation kinetics of small non-coding RNA in bacteria

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Small RNAs (sRNAs) play important roles in regulating gene expression through a variety of mechanisms. As one of the most common strategies, sRNA induced target messenger RNA (mRNA) includes two major steps: target search by base-pairing interactions with the and downstream execution by modulating translation or the stability of the mRNA. Here we describe a new imaging and analysis platform based on super-resolution fluorescence microscopy, which enabled the first in vivo kinetic measurement of sRNA-mediated gene regulation. Specifically, this platform was used to investigate a sugar-phosphate stress-induced bacterial sRNA that induces the degradation of target mRNAs. The data reveal that the sRNA binds to a primary target mRNA in a reversible and dynamic fashion, and that formation of the sRNA-mRNA complexes is the rate-limiting step, dictating the overall efficiency of regulation in vivo; whereas the downstream co-degradation of sRNA-mRNA complex can kinetically compete with the fast complex disassembly. Examination of a secondary target of this sRNA indicated that differences in the target search kinetics contribute to setting the regulation priority among different target mRNAs. This super-resolution imaging and analysis approach provides a conceptual framework that can be generalized to other sRNA systems and other target search processes.