Effects of Post-Translational Histone Modifications on Transcription Rate

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Introduction

The structural organization of DNA in eukaryotic cells is greatly implicated in the regulation of gene expression and thus cellular properties and behavior. At the most fundamental unit of this organization, ~147 bp of DNA wraps 1.7 times around a histone octamer core, forming a collective unit called the nucleosome. The positioning and occupancy of these nucleosomes around the promoter elements of genes is known to be a strong regulator of transcription in eukaryotic nuclei, and post-translational modifications (PTM's) to the protruding N-terminal tails of histone proteins are known to influence chromatin structure and thus gene expression; however, relatively little is known about the residual effect of histone PTM's on transcription rate.

Methods

Here, we present a novel method for measuring the transcription rate of chromatin with variable histone composition using an engineered DNA construct (TB6NR) consisting of:

- C. elegans promoter elements (Cep-1, MLP)
- High-affinity nucleosome positioning 601 sequences [1] flanked by linker DNA
- The Broccoli RNA aptamer [2], a more versatile derivative of the Spinach aptamer [3]

Fluorescence of the Broccoli aptamer upon completion of RNA transcripts in the presence of a fluorophore (DFHBI) will allow for the quantification of real-time transcription rates using common qPCR instruments, and repeated experiments using various histone PTM's will enable a comparison of the transcription rates of chromatin with variable histone composition. If successful, the data collected using this technique will offer insights into the effects of PTM's on transcription, ultimately allowing for more precise manipulation of transcriptional output and thus gene expression in living organisms.

References

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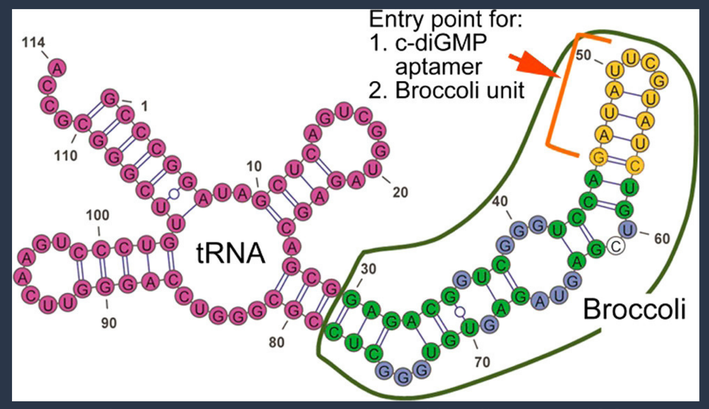

Broccoli aptamer structure as reported by Filonov et al.[2] Here, Broccoli is depicted attached to a tRNA scaffold for increased stability. For our purposes this scaffold would be unnecessary.

Transcription rate measurement using the novel Transcriptional Broccoli 601 Nucleosome Reconstitution (TB6NR) construct