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Detection and control of transcription initiation at the single-molecule level

Terence Strick, PhD
Cold Spring Harbor Fellow
Cold Spring Harbor Labs
Beckman Building
1 Bungtown Road
Cold Spring Harbor, NY 11724
<http://www.cshl.org/public/HT/ws01-staff.html>

The first step in gene expression is the transcription of a gene (DNA) into messenger RNA (mRNA) by RNA polymerase (RNAP). Gene transcription itself is a multistep process. RNAP first binds to a specific sequence (the promoter site) located just upstream of the gene. The polymerase then mechanically untwists or unwinds the promoter DNA so as to "open up" the DNA and gain intimate access to the basepairs that encode the gene. Next, upon addition of nucleotides (the building blocks of nucleic acids), the polymerase leaves the promoter site and begins transcribing DNA into mRNA. By monitoring the extension of a mechanically stretched, supercoiled DNA molecule containing a single bacterial promoter, we have been able to directly observe in real time the change in DNA extension associated with topological unwinding of ~1 helical turn of promoter DNA by RNAP. We have used the approach to quantify the extent of unwinding, the kinetics and stability of unwinding, and the effects of temperature, supercoiling, promoter sequence, effector ppGpp, and nucleotides. The rate of formation and the stability of the unwound promoter/RNAP complex depend strongly on supercoiling. Results indicate that RNA polymerase is extremely sensitive to the torque acting on the supercoiled DNA. Since DNA supercoiling is actively regulated *in vivo*, these results also suggest that DNA supercoiling could be used by the cell to regulate gene expression. Finally, this approach should be generalizable to any molecular motor causing changes in DNA twist and/or DNA compaction.