Transient DNA looping bridged on low-affinity sequences substantially promotes gene transcription

Yaolai Wang[1], Feng Liu[1,2] and Wei Wang[1]

***Short Abstract* — Although abundant structural data are available on the transcription apparatus (TA), little is known about how it operates kinetically. By analyzing the organization architectures of proteins binding to the regulatory DNA sequences, we first propose a model of how the TA operates on the *glnAp2* promoter. We then characterize the transcription behaviors in response to various concentrations of NtrC (transcription factor). Specifically, an enhancer and a low-affinity binding site can be transiently bridged by an NtrC oligomer at its low and intermediate levels, contributing to transcription initiation. This work also clarifies different roles for two enhancers in gene transcription.**

***Keywords* — transcription initiation, DNA looping, kinetics**

I. Background

Using a newly developed fluorescence technology, Friedman and Gelles recently dissected the major steps of transcription initiation at an activator-dependent bacterial promoter [1]. The study revealed the formation of two sequential closed complexes and an open complex, as well as the release of σ^{54}. Nevertheless, how the DNA-bound activators and the promoter-bound holoenzyme σ^{54}RNAP are positioned properly remains unclear. Here, we address this issue in terms of activity of the *glnAp2* promoter in *Escherichia coli*.

The *glnAp2* gene expresses the nitrogen assimilation enzyme glutamine synthetase under the regulation of NtrC (nitrogen regulatory protein C). Intracellular free NtrC molecules exist as dimers. Upon activation in nitrogen-starved cells, NtrC dimers are phosphorylated and can nucleate formation of hexamers. $NtrC_P$ binds to two strong sites (enhancers) at -140 (site I) and -108 (site II) and to three weaker sites at -89, -66 and -45 (sites III-V) relative to the transcription start site [2], while σ^{54}RNAP binds the -24~-12 region. It is generally held that enhancer-bound NtrC hexamers can contact and catalyze the holoenzyme, which then opens the DNA double strands and initiates mRNA synthesis. Whether and how those low-affinity sites also play a role is largely unknown.

Although the transcription apparatus (TA) on the *glnAp2* promoter involves only NtrC, σ^{54}RNAP and promoter DNA, it exhibits rather complex behaviors [2], some of which cannot be accounted for by traditional models. To elucidate how the TA operates, it is essential to associate the binding kinetics of NtrC with transcription initiation.

II. Results

By structurally and kinetically analyzing the organization architectures of NtrC and σ^{54}RNAP on the *glnAp2* gene, here we present a model of how the TA dynamically operates [3]. We propose that enhancer II and low-affinity site V can be transiently bridged by an enhancer II-bound NtrC tetramer/hexamer at low and intermediate activator concentrations. During the short lifetime of this conformation, another hexamer bound to enhancer I is just around the -24~-12 region; a newly recruited holoenzyme can be immediately activated to initiate mRNA synthesis, thus elevating transcriptional levels. At high concentrations of NtrC, the three low-affinity sites are occupied, rendering the DNA more rigid and hindering DNA bending to repress transcription. Stochastic simulation results further reproduce the experimental observations quantitatively. Experimentally testable predictions are also made.

III. Conclusion

A dynamic mechanism for transcription initiation on the *glnAp2* gene is provided, and weak molecular interactions can play a critical role in transcriptional regulation.

References

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[1] School of Physics, Nanjing University, Nanjing 210093, China.

[2] E-mail: fliu@nju.edu.cn.