

PROBING THE STRUCTURE OF THE DYNAMIC INITIAL TRANSCRIBING COMPLEXES ON *ESCHERICHIA COLI* PROMOTERS

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Transcription is the process by which the RNA polymerase binds to the DNA template and synthesizes RNA. This process is further divided into initiation, elongation and termination phases. During transcription initiation, the RNA polymerase (R) recognizes and binds to the promoter DNA (P) to form the closed complex, RPc. The double-stranded promoter DNA is then melted into single stranded transcription bubble, resulting in the open promoter-polymerase complex, RPo. Subsequently, in the presence of nucleotide substrate, the RNA Polymerase undergoes several rounds of abortive initiation before escaping from the promoter region. Thus, the abortive initiation-promoter escape reactions connect the initiation and elongation phases.

Previous investigation showed that depending on the initial transcribing sequences (ITS) of the promoter, the properties to which the polymerase achieves escape differs.¹ This was best illustrated with two *E.coli* $E\sigma^{70}$ promoters – N25 and N25anti, the latter contains the “anti” version of the ITS of the former. The ITS change did not affect their binding affinity to the RNA polymerase nor the rate of isomerization from RPc to RPo², but greatly altered their escape pattern as well as the rate of promoter escape.

In this project, I have determined their rates of promoter escape³ and have proceeded to examine the difference in their escape pattern. Specifically, the pattern differences refer to the fact that N25 undergoes escape at the +11/+12 junction while N25anti does so at the +15/+16 junction. The latter is the longest position of escape so far documented and predicts the existence of a very different population of initial transcribing complexes than on the N25 promoter. To capture this difference, we have employed various enzymatic and biochemical DNA footprinting methods⁴ to characterize the complex formation and the movement of the RNA polymerase along the DNA. We used DNaseI footprinting to find the protected region of the promoter DNA by the polymerase. KMnO₄ footprinting is also employed to determine the single stranded region of the DNA in open complexes versus initial transcribing complexes. Our goal is to illustrate and contrast the structures of the stable open complexes and the dynamic initial transcribing complexes for these two promoters.

¹ Hsu, LM, Cobb, IM, Ozmore, JR, Khoo, M, Nahm, G, Xia, L, Bao, Y, Ahn, C (2006) Initial transcribed sequence mutations specifically affect promoter escape properties. *Biochemistry* 29: 8841-54

² Kammerer W, Deuschle U, Gentz R, Bujard H (1986) Functional dissection of *Escherichia coli* promoters: information in the transcribed region is involved in late steps of the overall process. *EMBO J.* 5: 2995-3000

³ Aye-Han, N, Hsu, LM. Initial transcribed sequence and template conformation affect the kinetics of promoter escape. Poster Presented at Molecular Genetics of Bacteria and Phages Meeting, Cold Spring Harbor, New York, August 2006.

⁴ Zaychikov, E, Denissova, L, Heumann, H (1995) Translocation of the *Escherichia coli* transcription complex observed in the registers 11 to 20: “Jumping” of RNA polymerase and asymmetric expansion and contraction of the “transcription bubble”. *Proc. Natl. Acad. Sci. USA* 92: 1739-1743