Functional analysis of spirochetal promoters using a reconstituted transcription system

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BIOCHEMISTRY

INTRODUCTION

RNA polymerase can read regulatory signals encoded in the genomic DNA and respond to the changes in the concentration of substrate NTPs. However, most of the regulatory inputs are delivered to RNA polymerase via accessory protein factors and regulatory RNAs.

In the initiation of transcription RNA polymerase binds to a promoter. The promoter has up to four main elements: UP-element, -35, extended -10 and -10 regions (Figure 1). RNA polymerase needs a sigma factor for proper promoter binding. After -10 region there is usually a six base pair spacer followed by the starting base (+1) of transcription.

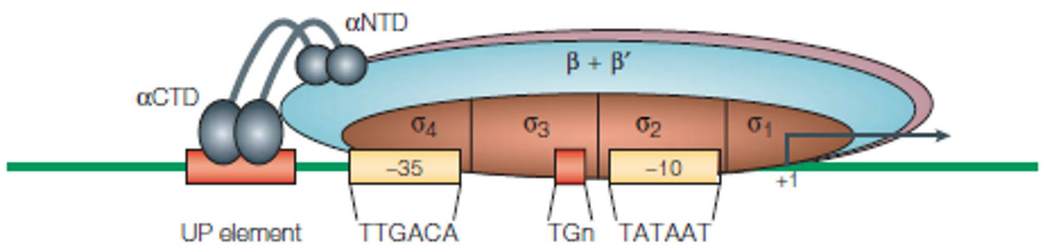

Figure 1. (Browning & Busby 2004) RNA polymerase holoenzyme bound to a promoter.

The environment of different bacteria species differ a lot from each other. *Spirochaeta africana* is alkalophilic bacteria which optimal growth pH is close to 9.

MATERIALS AND METHODS

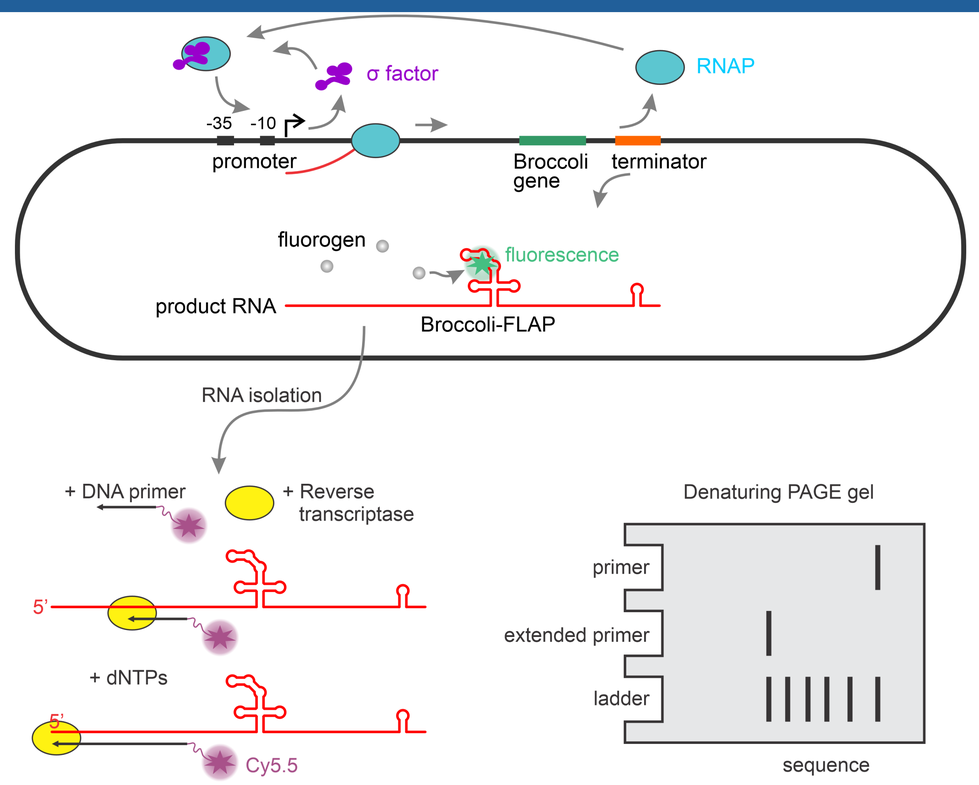

Figure 2. (Huang et al. 2022 modified) Spirochaetal transcription output was monitored at both pH 7.5 and pH 9 by following the fluorescence levels of the folded RNA aptamer bound to a fluorogen. In addition, transcription factor CarD was added to experimental setup with selected promoters. Using the same experimental setup RNA was isolated after transcription for primer extension assay to map the transcription start sites for a subset of promoters to confirm their identities with Escherichia coli and S. africana RNA polymerases.

RESULTS

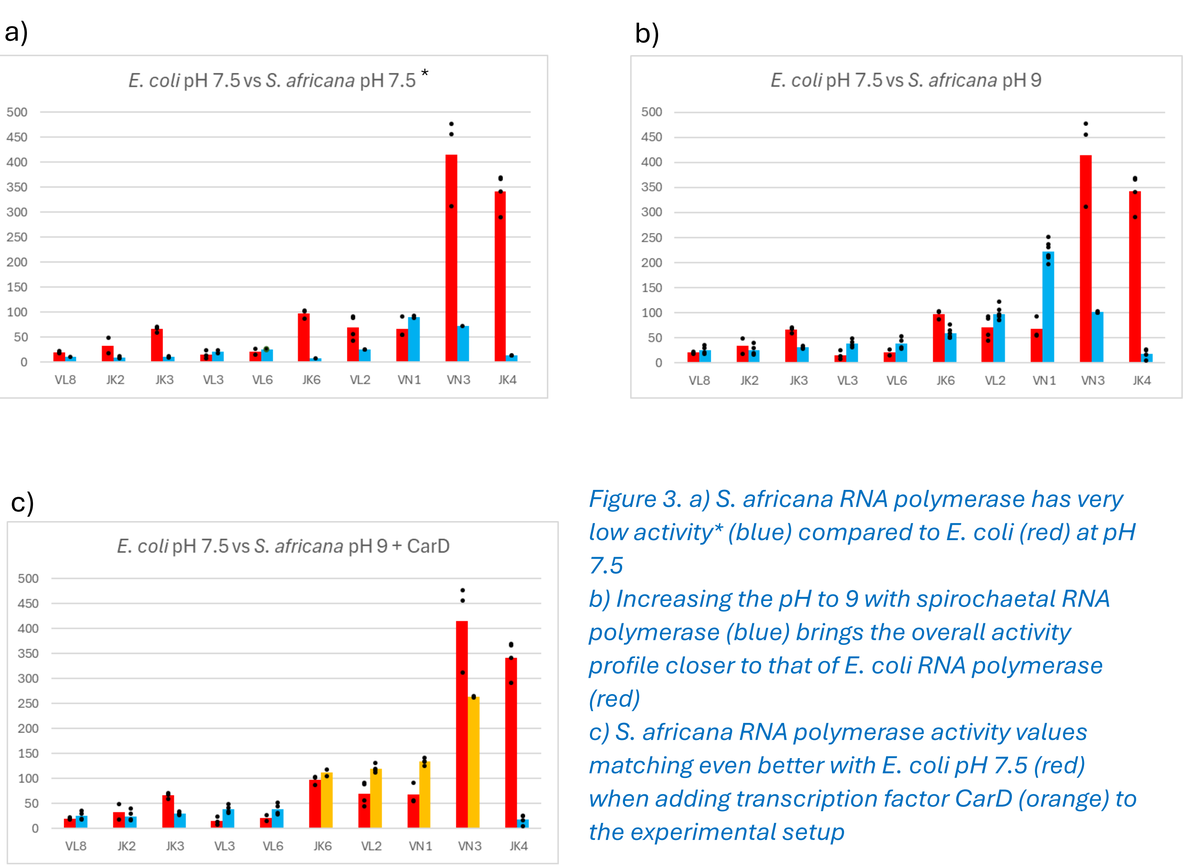

Figure 3. a) S. africana RNA polymerase has very low activity (blue) compared to E. coli (red) at pH 7.5*
b) Increasing the pH to 9 with spirochaetal RNA polymerase (blue) brings the overall activity profile closer to that of E. coli RNA polymerase (red)
c) S. africana RNA polymerase activity values matching even better with E. coli pH 7.5 (red) when adding transcription factor CarD (orange) to the experimental setup

The activity profile of spirochaetal RNA polymerase matched the *E. coli* RNA polymerase profile best when the former enzyme was assayed at pH 9 in the presence of CarD (Figure 3).

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          -35                      -10
IP4     TTGACGAAACTCGTTAACATGTTATAATACAAGCATCGAGAGGGACACGAGGAACCAGAGGAGTT
JK6   TTGACAGCAAAATCGATGTCTGCTATATTTCGCGAACAGTTCACCAGCCCCTGTAGCTCAGCGGTA
VN1     TTGACGAAACTCGTTAACATGTTATAATACAAGCGTTACCATAGTTGAATAACAATCTACGACTC
VN3   TTGACCAAAGCAGGCGAGTCGACTATATTCACGATCACGCGCCGCGAGGCGCCGATCGCGAGTCAG
VL2   TTGACACGGTGCCATCCATTATTATGGTGTTGGTTCAAGTATATACCCCTCAGGAGGTCATGTGG
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Figure 4. Aligned promoter sequences. -35 and -10 regions are marked on yellow, and the predicted start site of transcription is marked on green.

To map the starting sites of the selected promoters used in transcription activity assays RNA was isolated after transcription and converted to cDNA with reverse transcriptase and fluorescent primers (Figure 2). cDNA samples were run in a denaturing PAGE gel with a ladder to define its length (Figure 5). That was used to mark the starting base of transcription. The predicted starting base of the promoter according the sequence alignment (Figure 4) and the corresponding base of cDNA (Figure 5) are marked on green background. The starting site of transcription was defined in the assay with *E. coli* RNA polymerase in 5a, c and d whereas *S. africana* RNA polymerase was used in b and e.

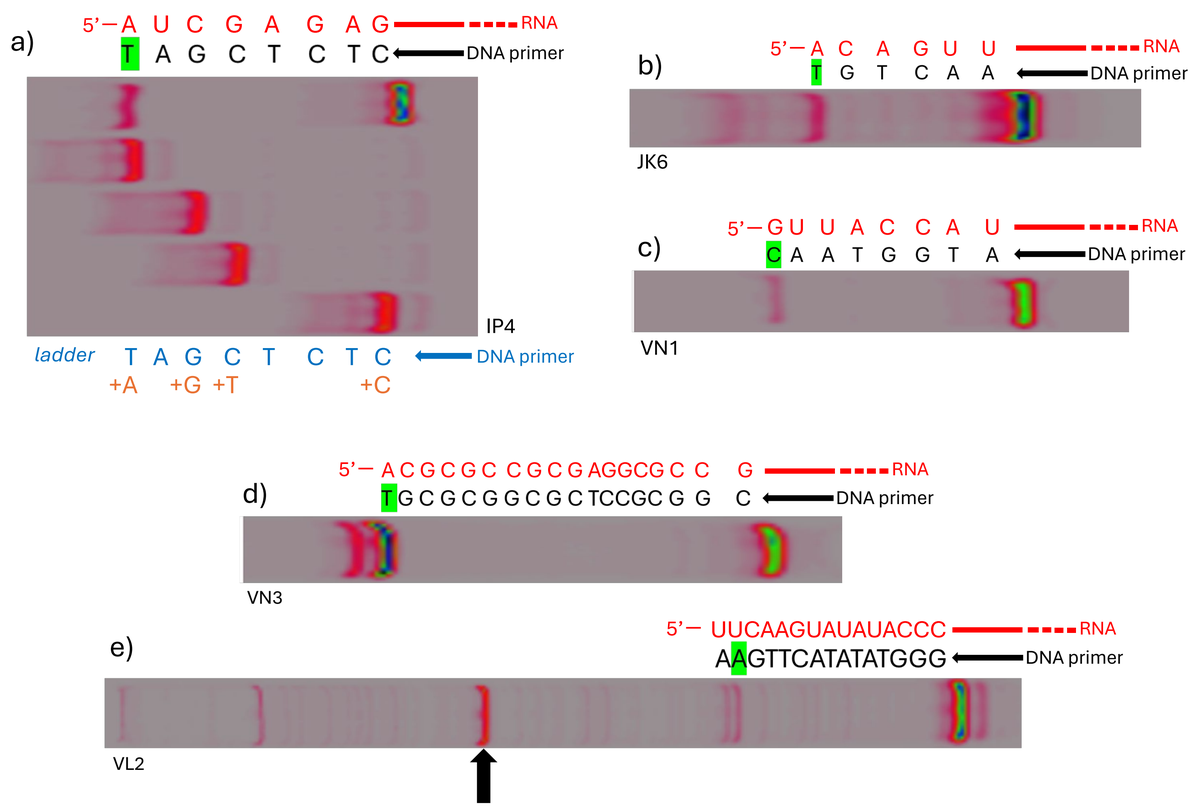

Figure 5. Gel images of cDNA with fluorescent primers run in a PAGE gel. The length of the cDNA was defined with a ladder presented in the gel a. The ladder (blue) was synthesized with DNA polymerase using a fluorescent primer and a DNA template. The different size DNA samples in the ladder were synthesized by adding nucleotides one by one for each reaction: first C, then C and T etc. (orange).

In all cases (Figures 5a, b, c, d) except one (e) the start site was as predicted according to sequence alignment. In Figure 5e is seen that *S. africana* RNA polymerase utilized a cryptic promoter *in vitro* that was distinct from the predicted promoter.

CONLUSIONS

S. africana RNA polymerase has likely evolved to operate at high pH close to the optimal extracellular pH for *S. africana* growth. In addition, CarD seems to be a global regulator of transcription in *S. africana*. *S. africana* promoters also differ markedly in their response to the increase in pH: some get activated several fold whereas others remain unaffected. Further mechanistic studies of pH-activatable promoters will further our understanding of the mechanism of transcription initiation in bacteria and its regulation.

References

Browning, D. F. & Busby, S. J. (2004) The regulation of bacterial transcription initiation. *Nat Rev Microbiol* **2**:57–65.

Huang, Y.-H., Trapp, V., Puro, O., Mäkinen, J. J., Metsä-Ketelä, M., Wahl, M. C. & Belogurov, G. A. (2022) Fluorogenic RNA aptamers to probe transcription initiation and co-transcriptional RNA folding by multi-subunit RNA polymerases. *Methods Enzymol* **675**:207–233.

*Transcription activity of *S. africana* at pH 7.5 was assayed and analyzed by Vilma Trapp