Principles Governing the Activity of *E. coli* Promoters

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1 Introduction

The control of transcription initiation is central among the mechanisms which regulate gene activity. In *E. coli* for example the rate of RNA synthesis directed by various promoters can differ by orders of magnitude. Moreover, the activity of an individual promoter may be modulated over a wide range by negatively and/or positively acting regulatory proteins. What are the principles which determine promoter activity and how can this activity be modified by regulatory proteins?

To serve as a transcriptional start signal for RNA polymerase a DNA sequence has to fulfill two basic requirements:

- it has to be localized by the enzyme within an abundance of unspecific sites in a biologically sensible time span and
- it has to encode a functional program which the enzyme can decipher and turn into biochemical events, i.e., the precise initiation of RNA synthesis and its template-dependent continuation.

Since the activity of a promoter is best defined by the rate at which a productively transcribing RNA polymerase leaves its start site, any step of the interaction between the enzyme and the promoter which precedes this event (Fig. 1) can in principle be rate-limiting and therefore determine promoter activity.

![Fig. 1](image_url)

**Fig. 1.** Pathway of RNA-polymerase promoter interaction. Upon encountering a promoter sequence RNA polymerase forms a complex in which the DNA is still in a “closed” state (*RPc*). By passing through intermediate states (*RPi*) the “open” complex (*RPo*) is established in which a stretch of 10 to 15 bp is melted. *RPo* is assumed to readily initiate RNA synthesis (*RPi*). Upon a drastic conformational change the enzyme clears the promoter engaged in the elongation complex (*RDNAel*)

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activity. Accordingly, promoters with identical in vivo activity should exist which, however, are limited at different steps of their interaction with RNA polymerase. Such promoters are not expected to be properly described by a single consensus sequence, instead their sequences might identify them as members of different promoter families whose functional program is optimized in alternate ways. Why should such promoter families exist at all? One argument for a wide spectrum of differently optimized promoters would be that it increases the possibilities for regulatory proteins to participate in a specific way in the interaction between promoter and RNA polymerase (RNAP), and first examples in support of this view will be discussed below.

Here we wish to summarize primarily work of our group which has largely been based on hypotheses as developed above and which was in particular concentrated on questions like the following:

Which are the limiting steps in the sequential pathway of the RNAP promoter interaction?
Are there promoters whose functional program reflects differently optimized pathways?
Can sequence elements of a promoter be correlated with partial functions and how do regulatory proteins exert their function during RNAP-promoter interaction?

Thus, we studied a panel of more than 20 promoters utilized by the $\sigma^{70}$ E. coli RNA polymerase of which some are strong phage promoters like PL, PA1, and PN25 from lambda, T7, and T5, respectively. It was hoped that especially these phage promoters would exhibit most clearly some pertinent features and would therefore facilitate their structural and functional analysis.

2 Promoter Activity in Vivo

Crucial for all our studies was the experimental system developed in our laboratory by U. Deuschle which permits to accurately determine the strength of promoters in vivo (Deuschle et al. 1986). Figure 2 depicts the principle of the assay by which the activity of a promoter is related to an internal standard promoter, here Pbla. It should be noted that rates of RNA synthesis are measured and that differences in mRNA half-life times were taken into account whenever necessary. The range of in vivo strengths found within the panel of promoters spans around two orders of magnitude and the hierarchy within this range reveals that some of the phage promoters like PA1, PH207 and PL are indeed very strong transcription initiation signals. This is supported by the comparison between the activity of PN25 and the rate of rRNA synthesis at logarithmic growth of the culture, which reveals that phage promoters like PA1 and PL produce transcripts with close to maximal rates (Deuschle et al. 1986). These data suggest furthermore that a single promoter sequence is capable of initiating productive transcription at optimal rates, i.e., around one transcript per second (Sarmientos and Cashel 1983). On the other hand, Pbla of the $\beta$-lactamase gene (derived from pBR322) generates a transcript only every 100 s, demonstrating that se-