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MECHANISM OF INITIATION OF TRANSCRIPTION IN *ESCHERICHIA COLI*

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Abstract: The mechanism of initiation of transcription in *E. coli* has been studied in detail by arresting the intermediates and analysing them using various biochemical techniques.

Regulation of gene expression takes place, in majority of cases, at the stage of initiation of transcription. In *E. coli*, transcription is brought about by single enzyme, RNA polymerase which is composed of five subunits, viz., two 'alpha, beta, beta' and sigma (1). Of these, sigma leads to the correct promoter search, binds directly to the promoter sequences and hence, plays crucial role in the initiation of transcription (2). After the transcription is initiated, sigma is released from the elongating complex (3).

The promoters are unique DNA stretches that direct the correct sequence and amount of RNA being produced by the RNA polymerase (4). We have used an *in vitro* transcription system with promoters of various strengths and purified *E. coli* RNA polymerase. Our approach has been to arrest the process of initiation by limiting the NTPs present in the reaction mixture, isolate such intermediates and analyse them to dissect the molecular mechanism of the initiation of transcription.

We have observed that in addition to various other factors, DNA supercoiling has differential (promoter specific) influence on the initiation of transcription (5). Our results support that initiation of transcription is a multistep process(6) and that each step could be influenced either by promoter sequence, protein factors, antibiotics, ions

or environmental factors (temperature), providing handle for the regulation of the process.

Binding of RNA polymerase to the promoter and its subsequent translocation to elongation mode leading to promoter escape are the opposite events. Of these, promoter recognition is not involved in regulation while promoter escape is the regulatory event and is dependent on its strength. The general picture that emerges from our results is that transition from initiation to elongation mode, qualified by stable ternary complex formation (DNA - RNA polymerase - RNA) and release of sigma factor, are decisive for the promoter strength. "Earlier the transition, i.e., at smaller RNA length stronger the promoter" seems to be the trend.

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