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Review

Promoter-proximal pausing and its release: Molecular mechanisms and physiological functions

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ABSTRACT

For a long time, not much attention had been paid to post-initiation steps in transcription, because it was widely believed that transcriptional control was brought about almost entirely through the regulation of transcription initiation. However, it has become clear that the process of elongation is also tightly controlled by a collection of regulatory factors called transcription elongation factors and contributes, for example, to rapid induction of immediate-early genes and to the control over the viral life cycle. Transcription elongation has attracted attention also because this process is coupled with various RNA processing events. In this review, we discuss biochemical and physiological aspects of elongation control, particularly focusing on the role of the negative elongation factor NELF.

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A general overview of transcription elongation

Transcription can be divided into at least four steps. First, a set of general transcription factors and RNA polymerase II (Pol II) are assembled onto the promoter region of a gene to form a preinitiation complex (preinitiation complex assembly). Second, double-stranded DNA around the transcription start site (TSS) becomes partially melted, and RNA synthesis is initiated (transcription initiation). Then, Pol II leaves the promoter region and tracks along DNA to synthesize long nascent RNA (elongation). When Pol II reaches the 3′ end of the gene, Pol II becomes dissociated from DNA and RNA (termination).

Transcription elongation is a discontinuous process that is often interrupted for various reasons. During elongation, Pol II has four choices: (i) continuing RNA synthesis, (ii) pausing, (iii) backtracking, and (iv) termination. Backtracking is backward translocation of Pol II on the template, by which the 3′ end of nascent RNA is disengaged from the active site of Pol II. Backtracked Pol II is in a stably stalled condition, which is called arrest, and usually requires other protein factors to restart RNA synthesis. A group of proteins called transcription elongation factors play a key role in elongation control, as detailed below. These factors control elongation either positively or negatively, in most cases by directly interacting with Pol II.

In addition, histones have an important regulatory role in elongation control. Nucleosome works as a roadblock to Pol II and generally represses transcription elongation. It has also been shown that various histone modifications occur not only in the promoter region of a gene but also in the coding region. Although functional significance of histone modifications in the coding region remains largely unknown, the histone modifications may lead to the recruitment of positive elongation factors. Alternatively, the histone modifications may prevent aberrant transcription initiation from within the coding region.

The C-terminal domain (CTD) of Pol II

Pol II is a huge complex composed of 12 subunits, Rpb1 to Rpb12, with a molecular weight of over 0.5 MDa. The C-terminal domain (CTD) of the largest subunit Rpb1 has a heptapeptide motif, Tyr-Ser-Pro-Thr-Ser-Pro-Ser, which is repeated 26 times in yeast and 52 times in humans. Although all the three RNA polymerases have the same evolutionary origin and have similar subunit compositions, the CTD is unique to Pol II. Right after the cloning of Rpb1 and the discovery of the CTD in the 1980s, it was postulated that the CTD plays an important, Pol II-specific role. Subsequent studies have shown that the CTD is indeed involved in many of the mRNA synthesis steps, including transcription activation; transcription initiation, elongation, and termination; and mRNA capping, splicing, and 3′ processing [1]. Although mRNA processing was once considered largely as a post-transcriptional process, it has become clear that mRNA processing is in fact coupled with transcription spatiotemporally. Buratowski coined the term “CTD code” to describe the diverse role played by the Pol II CTD [2]. Although the CTD code may not be as complex as the histone code, the CTD is subject to various types of chemical modifications, and functional roles of Ser-2, Ser-5, and Ser-7 phosphorylations have been established clearly. On the one hand, Ser-5 and Ser-7 of the CTD are mainly phosphorylated by the kinase component of the general transcription factor TFIIH around the TSS. On the other hand, Ser-2 of the CTD is mainly phosphorylated by the transcription elongation factor P-TEFb (positive transcription elongation factor b) downstream of the TSS during processive elongation. A number of molecules “reading” the CTD code have been identified: some interact with the CTD in a phosphorylation-dependent manner, and some in a phosphorylation-sensitive manner.

Promoter-proximal pausing controlled by DSIF, NELF, and P-TEFb

DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole), first described in the 1950s as a synthetic inhibitor of the multiplication of several viruses, is a nucleoside analog that inhibits Pol II transcription. In the presence of DRB, synthesis of pre-mRNAs and mRNAs is strongly inhibited, and instead, short transcripts are accumulated. Transcription inhibition by DRB could be reproduced in vitro by using crude cell extract, but not when a highly purified transcription system was employed, which suggested that the target of DRB is not Pol II itself but a factor that plays an important role in mRNA synthesis through regulation of the elongation process. Price and Handa laboratories independently characterized the mechanism of action of DRB and identified novel elongation factors responsible for DRB action in the 1990s [3–5].

P-TEFb was purified and identified from *Drosophila* Kc cells as a factor that stimulates transcription elongation in a DRB-sensitive manner. P-TEFb is the DRB-sensitive protein kinase composed of Cdk9 and Cyclin T. On the other hand, DSIF (DRB sensitivity-inducing factor) and NELF (negative elongation factor) were purified and identified from human HeLa cells as factors that mediate DRB-induced transcription inhibition. DSIF is composed of Spt4 and Spt5, which were originally isolated in yeast by a genetic screen for regulators of transcription initiation sites [6]. NELF is composed of four subunits, A, B, C/D and E. NELF and P-TEFb are, as their names suggest, involved in repression and activation of transcription elongation, respectively. On the other hand, DSIF exerts dual regulatory effects by stably interacting with Pol II and recruiting various other factors to the Pol II elongation complex. Soon after transcription initiation, DSIF and NELF become associated with Pol II and induce its pausing around 25 to 50 nucleotides downstream of the TSS (Fig. 1). This is a general rate-limiting step of transcription called promoter-proximal pausing. In a paused state, Ser-5 of the Pol II CTD, but not its Ser-2, appears to be phosphorylated already [7]. Ser-2 phosphorylation by P-TEFb results in a release of the pause by causing the dissociation of NELF from Pol II (Fig. 1) [5]. Thus, DRB inhibits the early step of transcription elongation by inhibiting the P-TEFb kinase.

P-TEFb also phosphorylates NELF-E and the C-terminal region (CTR) of Spt5, the large subunit of DSIF (Fig. 1). The Spt5 CTR has a repetitive structure similar in sequence to the Pol II CTD and appears to function within the elongation complex as another scaffold for regulatory proteins. In any case, phosphorylation of the Spt5 CTR converts DSIF from a repressor to an activator [8]. After CTR phosphorylation, DSIF recruits two other transcription elongation factors, the Paf1 complex (Paf1C) and Tat-SF1, to the Pol II elongation complex, and these factors together facilitate