Review Article

Control of Transcriptional Elongation by RNA Polymerase II: A Retrospective

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The origins of our current understanding of control of transcription elongation lie in pioneering experiments that mapped RNA polymerase II on viral and cellular genes. These studies first uncovered the surprising excess of polymerase molecules that we now know to be situated at the 5′ ends of most genes in multicellular organisms. The pileup of pol II near transcription start sites reflects a ubiquitous bottle-neck that limits elongation right at the start of the transcription elongation. Subsequent seminal work identified conserved protein factors that positively and negatively control the flux of polymerase through this bottle-neck, and make a major contribution to control of gene expression.

1. Introduction

The initiation phase of the RNA polymerase II (pol II) transcription cycle involves multiple events, including recruitment of general transcription factors and pol II to the promoter, melting of the DNA template, initiation of RNA synthesis, and pol II promoter clearance, which marks entry into the elongation phase. The stochastic nature of all of these steps poses a potential problem if it becomes necessary to mount a rapid activation of transcription. Following initiation pol II often encounters a rate-limiting barrier that appears to lie between early elongation and productive elongation. The transition between these two phases of the transcription cycle has now been characterized as a powerful regulatory switch used to increase or decrease gene expression in a signal-responsive fashion. Here we review the early discoveries that laid the foundation for a detailed understanding of transcriptional regulation at this transition.

2. Early Evidence of Polymerase Pausing and Premature Termination in DNA Viruses

Nearly 30 years ago it was reported by the late Yosef Aloni and colleagues that run-on transcripts made in nuclei from SV40 infected cells were strongly biased toward the 5′ end of the late transcription unit suggesting that pol II accumulated in the promoter-proximal region [1]. Analysis of labeled RNA extended on viral transcription complexes (VTCs) assembled in vivo and purified from infected cells revealed two additional unusual features of transcription from the late promoter. First, two pause sites were mapped around positions +15 and +40 relative to the start site by identifying the junctions between unlabelled RNA made in vivo and labeled RNA extended in vitro [2]. Second, a major product of transcription on VTCs is a discrete 93–95 base RNA, that is, prematurely terminated near a potential hairpin loop structure. Similar evidence for promoter-proximal stalling and/or premature termination were subsequently reported for the early and late promoters of polyoma virus [3]. These results prompted speculation that SV40 late transcription might be regulated by a mechanism [1] that regulates a decision between premature termination and productive elongation, analogous to attenuation on bacterial operons [4]. About the same time Luse and colleagues showed that transcription complexes assembled in HeLa nuclear extract on the adenovirus 2 major late promoters under NTP limiting conditions gave rise to uncapped transcripts about 20 nucleotides long that could be elongated into capped transcripts.
It was not long before the first evidence emerged that pol II was distributed across so many genes. The original in vitro pulse chase experiments of Coppola and colleagues showed that pol II can pause close to the start site and then resume elongation [5]. Since then, the most popular interpretation of in vivo polymerase mapping studies has been that they result from a similar “promoter-proximal pausing” phenomenon. That at least some promoter-proximal polymerase can resume elongation is demonstrated by nuclear run-on experiments; indeed, these polymerases would not be detectable by this method if they could not elongate and incorporate labeled nucleotides. However, the possibility that some fraction of the promoter-proximal polymerases terminate prematurely and never enter the productive elongation phase cannot be eliminated. The evidence for premature termination is quite clear for the SV40 late and HIV viral genes [24, 25], but it is much less compelling for cellular genes. Prematurely terminated RNAs are a major product of c-myc transcription in microinjected Xenopus oocytes, but the physiological relevance of this phenomenon remains unproven [26]. Recently, short (20–90 bases) transcription start site-associated (TSS-a) sense and antisense transcripts present at very low levels in the nucleus were detected by high-throughput RNA sequencing [27]. Whether these transcripts are products of promoter-proximal premature termination or pol II pausing are interesting questions for future investigation.

4. Promoter-Proximal Pausing versus Premature Termination

What is the root cause for why pol II is so unevenly distributed across so many genes? The original in vitro pulse chase experiments of Coppola and colleagues showed that pol II can pause close to the start site and then resume elongation [5]. Since then, the most popular interpretation of in vivo polymerase mapping studies has been that they result from a similar “promoter-proximal pausing” phenomenon. That at least some promoter-proximal polymerase can resume elongation is demonstrated by nuclear run-on experiments; indeed, these polymerases would not be detectable by this method if they could not elongate and incorporate labeled nucleotides. However, the possibility that some fraction of the promoter-proximal polymerases terminate prematurely and never enter the productive elongation phase cannot be eliminated. The evidence for premature termination is quite clear for the SV40 late and HIV viral genes [24, 25], but it is much less compelling for cellular genes. Prematurely terminated RNAs are a major product of c-myc transcription in microinjected Xenopus oocytes, but the physiological relevance of this phenomenon remains unproven [26]. Recently, short (20–90 bases) transcription start site-associated (TSS-a) sense and antisense transcripts present at very low levels in the nucleus were detected by high-throughput RNA sequencing [27]. Whether these transcripts are products of promoter-proximal premature termination or pol II pausing are interesting questions for future investigation.
a key difference between the activated and nonactivated states: the ratio of polymerases within the gene body relative to the 5′ end increased when transcription was activated. The significance of these studies is that they showed regulation of gene expression can be exerted at the level of transcriptional elongation by controlling the fraction of polymerases that are permitted to travel beyond the promoter-proximal region. Furthermore at Hsp70, the amount of paused pol II prior to heat shock correlated with the amount of mRNA made after heat shock [23]. Therefore, a satisfying answer to the question of why pol II accumulates near start sites is that it provides a pool of engaged polymerases ready for rapid mobilization in response to a gene activation stimulus. A second way that localized pol II accumulation at the TSS may enhance rapid transcriptional responses is excluding nucleosomes, thereby providing a bookmark in the chromatin that can be easily accessed by the transcriptional machinery [22]. A third suggestion is that an extended pol II dwell time within the promoter proximal region allows for cotranscriptional capping of the nascent mRNA [28, 29], and could help to “license” productive elongation complexes by allowing time for recruitment of processing and elongation factors. On the other hand, there is no direct evidence that a pol II pile-up near the TSS is required for efficient capping.

6. Control of Elongation by Transcriptional Activators

How is the flux of pol II from the promoter-proximal region into the body of a gene controlled? The first important clue was again provided by a virus; in this case HIV1. Groundbreaking work of Kao and colleagues showed that the viral transactivator protein Tat had the novel ability stimulate elongation by pol II [24]. Without Tat, most polymerases that initiate from the HIV1 LTR terminate prematurely shortly downstream of the TAR hairpin loop structure in a manner resembling the SV40 late transcription unit, but in the presence of Tat, pol II acquires the ability to extend transcripts all the way to the end of the provirus. To explain these surprising results, Kao et al. suggested that Tat regulates transcription by an antitermination mechanism similar to that exerted by the bacteriophage lambda protein N [30]. However, it remained possible that Tat also controlled transcriptional pausing, which is frequently a pre-requisite for termination.

HIV Tat is an unusual transactivator because it binds to the nascent DNA transcript. Therefore, the question remained open as to whether conventional DNA-bound activators can influence transcriptional elongation. Part of the answer to this question came with the demonstration that Tat could activate transcription when tethered to a DNA-binding site in the promoter [31]. Subsequent studies showed that enhancers and promoter-bound chimeric transcription factors comprising activation domains fused to a DNA-binding domain can stimulate elongation [32]. Furthermore a number of natural cellular activators stimulate elongation including heat-shock factor, NFkB, and c-myc [21, 33, 34]. Activation domains that enhance elongation and initiation, respectively, can synergize with one another and the most potent activation domains such as Herpes virus VP16 can stimulate both initiation and elongation [35, 36].

7. The Yin and Yang of Elongational Control

How do activators like HIV Tat and cellular transcription factors stimulate pol II transit away from the promoter-proximal region and into the downstream region of the gene for productive mRNA synthesis? The solution to this problem was provided by landmark studies that uncovered novel inhibitors of elongation and the factors that antagonize them. This story started with an early insight into how the ATP analogue 5, 6-dichloro-1-ß-D-ribofuranosylbenzimidazole (DRB) inhibits pol II transcription. Pulse labeling of RNA in adenovirus-infected cells revealed that DRB inhibited chain elongation but not initiation [37]. In a tour de force of classical biochemistry, the Handa and Price labs took advantage of this inhibitor to identify the core negative and positive factors that control the “yin and yang” of transcriptional elongation. Handa’s lab identified the DRB-sensitivity-inducing factor (DSIF) as Spt4/5 a conserved pol II binding complex that is required for inhibition of elongation near 5′ ends [38]. Soon afterwards, these workers identified a second negative-elongation factor, NELF, that cooperates with DSIF [39]. The counterpart to these negative factors is positive transcription elongation factor b (PTEFb) discovered by Marshall and Price [40]. PTEFb was identified as the cyclin-dependent protein kinase complex Cdk9-CyclinT1 [41, 42] that is specifically inhibited by DRB. In a remarkable convergence of independent studies, it turned out that the negative-factors DSIF and NELF and the positive-factor PTEFb are all components of the same control system. Thus, a major function of PTEFb is to “alleviate” the negative effects of DSIF and NELF [43] which it does by phosphorylating them both as well as the pol II C-terminal domain [44, 45].

Elucidation of the interplay between positive- and negative-elongation factors provided a basis for understanding how transcription factors can regulate elongation. The vital missing piece of the puzzle was filled in with the discovery that Tat when bound to TAR in the nascent HIV1 transcript contacts PTEFb through Cyclin T1 and this interaction is required for stimulation of transcriptional elongation [41, 42, 46]. Tat-mediated recruitment of PTEFb permits modification of the paused pol II complex by phosphorylation of the pol II CTD, Spt5, and NELF resulting in a transition to productive elongation. A similar mechanism involving PTEFb-mediated antagonism of the negative-elongation factors DSIF and NELF is thought to regulate elongation at many cellular genes including c-fos and NFkB targets [45, 47]. PTEFb (Cdk9/CyclinT1) is found embedded in multiple complexes with different protein and RNA subunits [48, 49] and there are likely to be multiple ways that it can be recruited to genes. These include binding directly to transcription factors [33] and chromatin components [50].

8. Concluding Remarks

Tremendous advances have been made in understanding control of gene expression at the level of transcriptional
elongation since the early days when it was identified on a few viral and cellular genes. Now this mechanism is recognized to be at least as important as control of the initiation step in pol II transcription. Still, important questions remain unresolved about the nature of promoter-proximally accumulated pol II. It is still not clear how many of these paused polymerases have backtracked and are destined ultimately to resume elongation and how many are destined for premature termination. These scenarios suggest the possibility of distinct targets for regulation by controlled polymerase release into the body of the gene. It will be interesting to see how these targets might be used in various developmental and signal-responsive contexts.

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References

[1] N. Hay, H. Skolnik-David, and Y. Aloni, “Attenuation in the control of SV40 gene expression,” Cell, vol. 29, no. 1, pp. 183–193, 1982.
[2] H. Skolnik-David and Y. Aloni, “Pausing of RNA polymerase molecules during in vivo transcription of the SV40 leader region,” EMBO Journal, vol. 2, no. 2, pp. 179–184, 1983.
[3] W. C. Skarnes, D. C. Tessier, and N. H. Acheson, “RNA polymerases stall and/or prematurely terminate nearby both early and late promoters on polyomavirus DNA,” Journal of Molecular Biology, vol. 203, no. 1, pp. 153–171, 1988.
[4] C. Yanofsky, “Transcription attenuation: once viewed as a novel regulatory strategy,” Journal of Bacteriology, vol. 182, no. 1, pp. 1–8, 2000.
[5] J. A. Coppola, A. S. Field, and D. S. Luse, “Promoter-proximal pausing by RNA polymerase II in vitro: transcripts shorter than 20 nucleotides are not capped,” Proceedings of the National Academy of Sciences of the United States of America, vol. 80, no. 5, pp. 1251–1255, 1983.
[6] D. S. Gilmour and J. T. Lis, “RNA polymerase II interacts with the promoter region of the noninduced hsp70 gene in Drosophila melanogaster cells,” Molecular and Cellular Biology, vol. 6, no. 11, pp. 3984–3989, 1986.
[7] A. E. Rougvie and J. T. Lis, “The RNA polymerase II molecule at the 5’ end of the uninduced hsp70 gene of D. melanogaster is transcriptionally engaged,” Cell, vol. 54, no. 6, pp. 795–804, 1988.
[8] D. L. Bentley and M. Groudine, “A block to elongation is largely responsible for decreased transcription of c-myc in differentiated HL60 cells,” Nature, vol. 321, no. 6071, pp. 702–706, 1986.
[9] D. Eick and G. W. Bornkamm, “Transcriptional arrest within the first exon is a fast control mechanism in c-myc gene expression,” Nucleic Acids Research, vol. 14, no. 21, pp. 8331–8346, 1986.
[10] C. Giardina, R. M. Perez, and J. T. Lis, “Promoter melting and TFIIID complexes on Drosophila genes in vivo,” Genes and Development, vol. 6, no. 11, pp. 2190–2200, 1992.
[11] A. E. Rougvie and J. T. Lis, “Postinitiation transcriptional control in Drosophila melanogaster,” Molecular and Cellular Biology, vol. 10, no. 11, pp. 6041–6045, 1990.
[12] Z. Chen, M. L. Harless, D. A. Wright, and R. E. Kellem, “Identification and characterization of transcriptional arrest sites in exon 1 of the human adenosine deaminase gene,” Molecular and Cellular Biology, vol. 10, no. 9, pp. 4555–4564, 1990.
[13] M. A. Collart, N. Tourkine, D. Belin, P. Vassalli, P. Jeanteur, and J. M. Blanchard, “c-fos Gene transcription in murine macrophages is modulated by a calcium-dependent block to elongation in intron 1,” Molecular and Cellular Biology, vol. 11, no. 5, pp. 2826–2831, 1991.
[14] J. Mirkovich and J. E. Darnell, “Mapping of RNA polymerase on mammalian genes in cells and nuclei,” Molecular Biology of the Cell, vol. 3, no. 10, pp. 1085–1094, 1992.
[15] L. J. Schilling and P. J. Farnham, “Inappropriate transcription from the 5’ end of the murine dihydrofolate reductase gene masks transcriptional regulation,” Nucleic Acids Research, vol. 22, no. 15, pp. 3061–3068, 1994.
[16] G. F. Crouse, E. J. Leys, and R. N. McEwan, “Analysis of the mouse dhfr promoter region: existence of a divergently transcribed gene,” Molecular and Cellular Biology, vol. 5, no. 8, pp. 1847–1858, 1985.
[17] A. Krumm, L. B. Hickey, and M. Groudine, “Promoter-proximal pausing of RNA polymerase II defines a general rate-limiting step after transcription initiation,” Genes and Development, vol. 9, no. 5, pp. 559–572, 1995.
[18] M. G. Guenther, S. S. Levine, L. A. Boyer, R. Jaenisch, and R. A. Young, “A chromatin landmark and transcription initiation at most promoters in human cells,” Cell, vol. 130, no. 1, pp. 77–88, 2007.
[19] G. W. Muse, D. A. Gilchrist, S. Nechaev et al., “RNA polymerase is poised for activation across the genome,” Nature Genetics, vol. 39, no. 12, pp. 1507–1511, 2007.
[20] L. J. Core, J. J. Waterfall, and J. T. Lis, “Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters,” Science, vol. 322, no. 5909, pp. 1845–1848, 2008.
[21] S. A. Brown, A. N. Imbalzano, and R. E. Kingston, “Activator-dependent regulation of transcriptional pausing on nucleosomal templates,” Genes and Development, vol. 10, no. 12, pp. 1479–1490, 1996.
[22] D. A. Gilchrist, G. Dos Santos, D. C. Fargo et al., “Pausing of RNA polymerase II disrupts DNA-specified nucleosome organization to enable precise gene regulation,” Cell, vol. 143, no. 4, pp. 540–551, 2010.
[23] H. S. Lee, K. W. Kraus, M. F. Wolfner, and J. T. Lis, “DNA sequence requirements for generating paused polymerase at the start of hsp70,” Genes and Development, vol. 6, no. 2, pp. 284–295, 1992.
[24] S. Y. Kao, A. F. Calman, P. A. Luciw, and B. M. Peterlin, “Anti-termination of transcription within the long terminal repeat of HIV-1 by tat gene product,” Nature, vol. 330, no. 6147, pp. 489–493, 1987.
[25] H. Skolnik-David, N. Hay, and Y. Aloni, “Site of premature termination of late transcription of simian virus 40 DNA: enhancement by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole,” Proceedings of the National Academy of Sciences of the United States of America, vol. 79, no. 9, pp. 2743–2747, 1982.
[26] D. L. Bentley and M. Groudine, “Sequence requirements for premature termination of transcription in the human c-myc gene,” Cell, vol. 53, no. 2, pp. 245–256, 1988.
[27] A. C. Seila, J. M. Calabrese, S. S. Levine et al., “Divergent transcription from active promoters,” Science, vol. 322, no. 5909, pp. 1849–1851, 2008.
[28] Y. Pei, B. Schwer, and S. Shuman, “Interactions between fission yeast Cdk9, its cyclin partner Pch1, and mRNA capping
enzyme Pct1 suggest an elongation checkpoint for mRNA quality control," _The Journal of Biological Chemistry_, vol. 278, no. 9, pp. 7180–7188, 2003.

[29] E. B. Rasmussen and J. T. Lis, "In vivo transcriptional pausing and cap formation on three Drosophila heat shock genes," _Proceedings of the National Academy of Sciences of the United States of America_, vol. 90, no. 17, pp. 7923–7927, 1993.

[30] J. Greenblatt, J. R. Nodwell, and S. W. Mason, "Transcriptional antitermination," _Nature_, vol. 364, no. 6436, pp. 401–406, 1993.

[31] C. D. Southgate and M. R. Green, "The HIV-1 Tat protein activates transcription from an upstream DNA-binding site: implications for Tat function," _Genes and Development_, vol. 5, no. 12, pp. 2496–2507, 1991.

[32] K. Yankulov, J. Blau, T. Purton, S. Roberts, and D. L. Bentley, "Transcriptional elongation by RNA polymerase II is stimulated by transactivators," _Cell_, vol. 77, no. 5, pp. 749–759, 1994.

[33] M. Barboric, R. M. Nissen, S. Kanazawa, N. Jabrane-Ferrat, and B. M. Peterlin, "NF-κB binds P-TEFb to stimulate transcriptional elongation by RNA polymerase II," _Molecular Cell_, vol. 8, no. 2, pp. 327–337, 2001.

[34] P. B. Rahl, C. Y. Lin, A. C. Seila et al., "C-Myc regulates transcriptional pause release," _Cell_, vol. 141, no. 3, pp. 432–445, 2010.

[35] W. S. Blair, R. A. Fridell, and B. R. Cullen, "Synergistic enhancement of both initiation and elongation by acidic transcription activation domains," _EMBO Journal_, vol. 15, no. 7, pp. 1658–1665, 1996.

[36] J. Blau, H. Xiao, S. McCracken, P. O’Hare, J. Greenblatt, and D. Bentley, "Three functional classes of transcriptional activation domains," _Molecular and Cellular Biology_, vol. 16, no. 5, pp. 2044–2055, 1996.

[37] N. W. Fraser, P. B. Sehgal, and J. E. Darnell, "DRB-induced premature termination of late adenovirus transcription," _Nature_, vol. 272, no. 5654, pp. 590–593, 1978.

[38] T. Wada, T. Takagi, Y. Yamaguchi et al., "DSIF, a novel transcription factor that regulates RNA polymerase II processivity, is composed of human Spt4 and Spt5 homologs," _Genes and Development_, vol. 12, no. 3, pp. 343–356, 1998.

[39] Y. Yamaguchi, T. Takagi, T. Wada et al., "NELF, a multisubunit complex containing RD, cooperates with DSIF to repress RNA polymerase II elongation," _Cell_, vol. 97, no. 1, pp. 41–51, 1999.

[40] N. F. Marshall and D. H. Price, "Control of formation of two distinct classes of RNA polymerase II elongation complexes," _Molecular and Cellular Biology_, vol. 12, no. 5, pp. 2078–2090, 1992.

[41] P. Wei, M. E. Garber, S. M. Fang, W. H. Fischer, and K. A. Jones, "A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA," _Cell_, vol. 92, no. 4, pp. 451–462, 1998.

[42] Y. Zhu, T. Peery, J. Peng et al., "Transcription elongation factor P-TEFb is required for HIV-1 Tat transactivation in vitro," _Genes and Development_, vol. 11, no. 20, pp. 2622–2632, 1997.

[43] T. Wada, T. Takagi, Y. Yamaguchi, D. Watanabe, and H. Handa, "Evidence that P-TEFb alleviates the negative effect of DSIF on RNA polymerase II-dependent transcription in vitro," _EMBO Journal_, vol. 17, no. 24, pp. 7395–7403, 1998.

[44] N. F. Marshall, J. Peng, Z. Xie, and D. H. Price, "Control of RNA polymerase II elongation potential by a novel carboxyl-terminal domain kinase," _The Journal of Biological Chemistry_, vol. 271, no. 43, pp. 27176–27183, 1996.

[45] T. Yamada, Y. Yamaguchi, N. Inukai, S. Okamoto, T. Mura, and H. Handa, "P-TEFb-mediated phosphorylation of hSpt5 C-terminal repeats is critical for processive transcription elongation," _Molecular Cell_, vol. 21, no. 2, pp. 227–237, 2006.

[46] H. S. Y. Mancebo, G. Lee, J. Flygare et al., "P-TEFb kinase is required for HIV Tat transcriptional activation in vivo and in vitro," _Genes and Development_, vol. 11, no. 20, pp. 2633–2644, 1997.

[47] L. Amir-Zilberstein, E. Ainbinder, L. Toube, Y. Yamaguchi, H. Handa, and R. Dikstein, "Differential regulation of NF-κB by elongation factors is determined by core promoter type," _Molecular and Cellular Biology_, vol. 27, no. 14, pp. 5246–5259, 2007.

[48] B. M. Peterlin and D. H. Price, "Controlling the Elongation Phase of Transcription with P-TEFb," _Molecular Cell_, vol. 23, no. 3, pp. 297–305, 2006.

[49] E. Smith, C. Lin, and A. Shilatifard, "The super elongation complex (SEC) and MLL in development and disease," _Genes and Development_, vol. 25, no. 7, pp. 661–672, 2011.

[50] Z. Yang, J. H. N. Yik, R. Chen et al., "Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4," _Molecular Cell_, vol. 19, no. 4, pp. 535–545, 2005.