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RNA Polymerase II Promoter-Proximal Pausing: Molecular Mechanisms of DSIF–NELF–Mediated Control of Early Elongation

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Promoter-proximal pausing of RNA Polymerase II (Pol II) is a key regulatory event in metazoan transcription, but its mechanistic underpinnings have been superficially treated in the literature. Pausing is, in essence, the stabilization of a specific early elongation complex by the elongation factors DSIF and NELF, in conjunction with the intrinsic properties of RNA Polymerase II, the nascent RNA, DNA sequence, and chromatin architecture. In this review, we seek to provide a comprehensive mechanistic analysis of RNA Polymerase II pausing, with special emphasis on how DSIF and NELF physically and kinetically reprogram the elongation complex to form a stable, reversible paused complex, and how the release of this paused complex is an irreversible event that marks the onset of productive elongation.

Specifically, we examine the early elongation phase following promoter escape, during which RNA Polymerase II is conformationally dynamic, susceptible to pausing, backtracking, abortive transcription, and abortive translocation, thereby creating an environment that is highly susceptible to regulatory influences that can bias the kinetic balance of the elongation complex towards productive elongation or paused states. We will review how DSIF interacts with both the RNA and various structural elements of the polymerase in a manner that limits polymerase movement, and how this process is further stabilized by the NELF complex in a manner that enforces promoter-proximal pausing. Finally, we will review how phosphorylation-dependent remodelling of the paused polymerase dismantles this checkpoint, linking the release of paused polymerases with cellular signalling, chromatin, and developmental events. This approach will provide a general understanding of promoter-proximal pausing as an "emergent behavior" of the polymerase that is actively regulated by elongation factors in a manner that fine-tunes gene expression in time and space.

Keywords: *Pol II pausing, NELF, DSIF, pause release, P-TEFb*

INTRODUCTION

Pausing as a Mechanistic Problem

Transcription by Pol II proceeds through a series of biochemically and structurally distinct intermediates. Initiation and termination have traditionally been acknowledged as regulatory nodes; however, the transition from initiation to productive elongation is now recognised as a critical checkpoint. Promoter-proximal pausing is characterised by Pol II initiating transcription and synthesizing a brief RNA, yet struggling to transition effectively into rapid, processive elongation.

From a mechanistic perspective, pausing is not merely characterised as “slow elongation” or as a passive impediment. The early elongation complex is stabilized, with its lifetime extended from seconds to minutes due to specific molecular interactions. A satisfactory model of pausing must explain (i) the intrinsic instability of early elongation, (ii) the selective stabilization of this instability by DSIF and NELF, and (iii) the mechanism of pause release without dismantling the transcription complex.

Early Elongation as an Intrinsically Unstable State

Subsequent to promoter escape, Pol II initiates early elongation while continuing to undergo significant conformational alterations. The transcription bubble is inadequately stabilized, the

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RNA–DNA hybrid is short, and the clamp and trigger loop exhibit various conformations (Cramer, 2019; Gnatt et al., 2001; Hahn, 2004). Single-molecule and biochemical investigations reveal that Pol II during this phase displays frequent stopping, backtracking, and abortive translocation occurrences (Galburt et al., 2007; Horn et al., 2014; Larson et al., 2012).

The promoter-proximal DNA sequence directly contributes to this instability. GC-rich sequences, asymmetric nucleotide distributions, and patterns that favor pauses enhance the probability of transitory backtracking (Kireeva, Kashlev, 2009; Larson et al., 2014; Pal, Luse, 2003). A single nucleotide backtrack displaces the RNA 3′ end from the active site, causing Pol II to become catalytically inactive until realignment or cleavage transpires (Cheung, Cramer, 2011; Kettenberger et al., 2004; Nudler, 2012). In the absence of regulatory considerations, such pauses are transient and swiftly rectified.

DSIF: Molecular Basis of Kinetic Restraint in Early Elongation

DSIF is the first elongation factor to interact with Pol II after promoter escape, and its function cannot be simplified to a binary positive or negative role. Mechanistically, DSIF modifies the early elongation complex by exerting kinetic constraints on a polymerase that is inherently unstable (Wada et al., 1998; Yamaguchi et al., 1999). Comprehending DSIF necessitates an examination of its structural design, recruitment time, and impact on Pol II translocation dynamics.

The SPT5 subunit of DSIF is a substantial, evolutionarily conserved protein characterised by several KOW domains that engage directly with the nascent RNA as it emerges (Dollinger et al., 2023; Meyer et al., 2015; Song and Chen, 2022). These RNA–protein interactions are not accidental; they anchor the transcript to the elongation complex and restrict its conformational flexibility. Simultaneously, SPT5 establishes significant interactions with the Pol II clamp, protrusion, and funnel domains, therefore successfully connecting RNA binding to the conformation of the polymerase (Klein et al., 2011; Li et al., 2014). SPT4 reinforces this contact by enhancing the SPT5–Pol II interface, hence prolonging the resident period of DSIF on the elongation complex (Uzun et al., 2021).

Functionally, DSIF modulates the kinetic partitioning of Pol II among forward translocation, stopping, and backtracking. In the first phase of elongation, Pol II often alternates between pre-translocated and post-translocated stages after nucleotide incorporation. DSIF prolongs the duration of these states by constraining clamp opening and reducing the flexibility of the bridge helix, both of which are essential for effective forward progression (Amith et al., 2025; Crickard et al., 2016). Consequently, Pol II advances at a reduced pace and more often adopts pause-prone conformations.

Importantly, DSIF does not cause a pause by inhibiting catalysis or obstructing the active site. Rather, it biases the elongation energy landscape, causing naturally induced pauses—arising from sequence context, RNA–DNA hybrid instability, or transitory backtracking—to last longer than they typically would (Dollinger et al., 2023). Without DSIF, these interruptions dissipate quickly, explaining why the depletion of DSIF results in the reduction of promoter-proximal Pol II accumulation, yet enhanced elongation downstream.

The contextual reliance of the DSIF function is crucial. During the initial elongation phase, when Pol II exhibits limited processivity and is susceptible to regulatory influences, DSIF acts as a kinetic inhibitor. Subsequent to the release of the pause, the phosphorylation of SPT5 by P-TEFb transforms DSIF into a positive elongation factor (Sun and Fisher, 2025). In this altered condition, the same RNA-binding and clamp-stabilizing characteristics inhibit backtracking and improve resistance to nucleosomal obstacles. Consequently, DSIF ought to be regarded as a molecular rheostat that modulates elongation behavior rather than only serving as an inhibitor.

NELF: Stabilization of a Backtracked, Poised Elongation Complex

NELF serves as the critical element that transforms a DSIF-slowed elongation complex into a stable, long-lasting paused state. In contrast to DSIF, which broadly modulates kinetics, NELF

functions with high specificity to immobilize Pol II in a catalytically inactive yet transcriptionally engaged state. This function relies on the sequential recruitment of NELF, its multivalent interactions, and its capacity to inhibit pause recovery pathways.

NELF is a heterotetramer, consisting of NELF-A, NELF-B, NELF-C/D, and NELF-E subunits (Yung et al., 2009). Each subunit provides unique interaction surfaces that together engage Pol II, DSIF, and the nascent RNA. Structural studies indicate that NELF is located near the RNA exit channel and secondary channel of Pol II, which are critical positions for regulating RNA positioning and the accessibility of elongation-associated factors (Richter, Taatjes, 2024; Vos et al., 2018). NELF recruitment depends on prior DSIF binding, indicating that NELF interacts with a DSIF-modified elongation complex rather than with free Pol II (Dollinger et al., 2023; Missra, Gilmour, 2010).

NELF stabilizes transcriptional pausing by sequestering Pol II in a partially backtracked or pre-translocated conformation. This configuration results in the displacement of the RNA 3′ end from the active site into the secondary channel, thereby inhibiting phosphodiester bond formation (Blears and Svejstrup, 2025). These states are often and briefly encountered by elongating Pol II but are typically resolved quickly through forward translocation or RNA cleavage. NELF significantly extends the duration of these states by physically restricting both the conformation of polymerase and the mobility of RNA (Su, Vos, 2024; Vos et al., 2018).

The RNA recognition motif (RRM) in NELF-E is essential for this stabilization. NELF-E binds to nascent RNA, thereby restricting the realignment of the RNA 3′ end with the active site and inhibiting spontaneous recovery from backtracking (Pagano et al., 2014; Rao et al., 2006). Simultaneously, NELF binding sterically and functionally opposes the recruitment or activity of transcript cleavage factors like TFIIS, which would otherwise facilitate pause escape by enhancing RNA cleavage (Kettenberger et al., 2003; Palangat et al., 2005). NELF effectively stabilizes the elongation complex in a paused state by inhibiting both forward and backward recovery pathways.

NELF does not disassemble the transcription complex. The DNA–RNA hybrid stays intact, the transcription bubble is sustained, and Pol II remains firmly attached to the template (Vos et al., 2018). This differentiates NELF-mediated pausing from transcriptional arrest or termination, ensuring that the paused complex retains full competency for rapid reactivation upon receiving appropriate signals.

Collectively, NELF functions as a molecular clamp that transforms kinetic instability into regulatory stability. Through the selective stabilization of a typically transient backtracked state, NELF establishes a paused population of Pol II that can endure for several minutes in vivo, thereby allowing for regulatory integration while maintaining transcriptional competence.

Cooperative Action of DSIF and NELF: Assembly of a Stable Paused State

Promoter-proximal pausing results not from the independent actions of DSIF or NELF, but from their ordered and cooperative interaction with Pol II. DSIF constructs a slow elongation regime that is prone to pauses, whereas NELF selectively stabilizes certain pauses into a long-lasting regulatory intermediate. This division of labor is crucial for the efficiency and reversibility of pausing.

The engagement of DSIF alone results in an increased frequency of pauses; however, it does not produce the distinct sharp peak of promoter-proximal Pol II that is typically observed across the genome. Without NELF, Pol II persists in sampling backtracked and pre-translocated states; however, these states quickly resolve via forward translocation or RNA cleavage. In contrast, NELF is unable to bind productively to Pol II that has not been kinetically constrained by DSIF (Wu et al., 2003; Yamaguchi et al., 2002). The paused state occurs solely when the kinetic slowing generated by DSIF coincides with the stabilization mediated by NELF.

This coordinated mechanism explains why the depletion of either of the two factors causes analogous phenotypes (no stable pausing and increased readthrough into gene bodies). Thus, it also

provides some insight into how the regulation of pause can be accomplished precisely but not at the expense of elongation efficiency. While DSIF remains associated with Pol II during elongation, NELF is associated with Pol II in a conditional, reversible manner; therefore, both temporal and spatial regulation of the length of pause can be achieved with precision.

Pause Release: Molecular Reprogramming Rather Than Simple Factor Loss

Pause release marks a critical and mainly irreversible shift in the life cycle of the transcription complex. This transition is facilitated by positive transcription elongation factor b (P-TEFb), a kinase complex consisting of CDK9 and cyclin T, whose activity is tightly regulated by sequestration and selective recruitment.

P-TEFb phosphorylates three primary substrates within the halted complex: the C-terminal domain (CTD) of Pol II at serine 2 residues of the heptad repeats, SPT5 in DSIF, and various subunits of NELF (Cheng and Price, 2007, p.; Fujinaga et al., 2004, p.; Marshall and Price, 1995). The phosphorylation processes are mechanistically interconnected. The phosphorylation of NELF diminishes its multivalent contacts with Pol II and the nascent RNA, resulting in the dissociation of the NELF complex (Dollinger, Gilmour, 2021; Fujinaga et al., 2004). The phosphorylation of SPT5 modifies its conformation, facilitating its progression, decreasing backtrack propensity, and enhancing its resilience to halt signals (Chen et al., 2020; Mayekar et al., 2013; Wier et al., 2013).

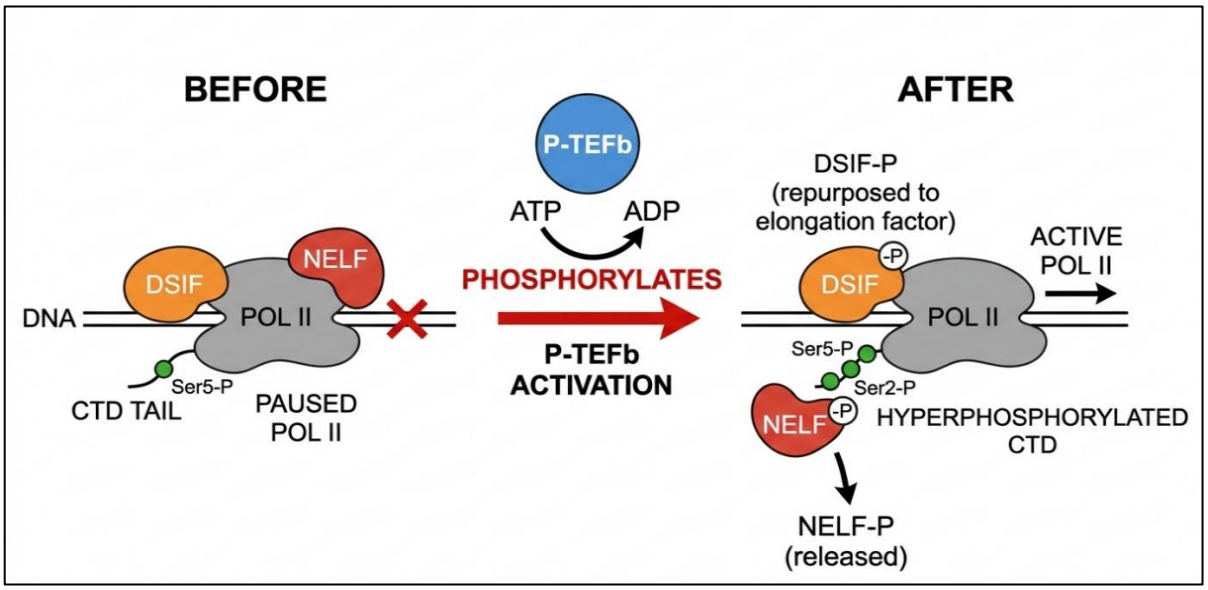


Figure 1: *Mechanism of P-TEFb-mediated RNA Polymerase II pause release.*

Transcription initiation leads to the formation of a paused RNA Pol II complex that is maintained by the negative elongation factors DSIF (orange) and NELF (red), with the Pol II CTD phosphorylated at Ser5. The pause is relieved by the kinase P-TEFb (blue) through the phosphorylation of three critical targets: (1) the negative elongation factor NELF, leading to its release from the complex, (2) DSIF, converting it to a positive elongation factor, and (3) the Pol II CTD, leading to the hyperphosphorylated form of the CTD. This allows the RNA Pol II complex to enter active productive elongation, leading to the synthesis of the nascent RNA transcript.

Phosphorylation of CTD Ser2 attracts a specific array of elongation and RNA-processing factors, including capping, splicing, and 3′ end processing mechanisms (Ahn et al., 2004; Davidson et al., 2014; Gu et al., 2013). This recruitment stabilizes the elongation complex and functionally commits Pol II to effective elongation. Following this reprogramming, the transcription complex ceases to display the kinetic features typical of the stopped state and does not re-engage in promoter-proximal pausing.

Chromatin Architecture as an Amplifier of Pausing

The chromatin environment is an important factor in the mechanism of and maintenance of promoter-proximal pausing. The +1 nucleosome, positioned immediately downstream of the transcription initiation site, poses a large energy barrier to elongation. The involvement of the +1 nucleosome increases the probability of backtracking and pausing of Pol II, especially during the first elongation phase (Abril-Garrido et al., 2023; Naganuma et al., 2025).

The DSIF-NELF-stabilized Pol II is highly susceptible to this energy barrier. The Pol II enzyme does not possess the ability to handle nucleosomes and translocate efficiently across the +1 nucleosome during paused states. After release from the paused state, phosphorylated DSIF and other elongation factors facilitate efficient disruption of histones and DNA and entry of Pol II into the gene. Hence, chromatin is not an initiator of pausing but is an enhancer of pausing and increases the probability of Pol II accumulation in the promoter-proximal region.

Histone marks of actively transcribed promoters, such as H3K4me3 and H3K27ac, are present in combination with transcriptional pausing (Li et al., 2025; Wang et al., 2023). This demonstrates that chromatin is an enhancer of pausing and facilitates the deposition of Pol II without influencing elongation.

Functional Consequences of Promoter-Proximal Pausing

Mechanistically stabilized pausing provides various regulatory benefits. Positioning Pol II downstream of the promoter in a transcriptionally engaged state allows pausing to bypass the rate-limiting steps of recruitment and initiation, facilitating rapid gene activation in response to signaling cues (Adelman, Lis, 2012; Gaertner, Zeitlinger, 2014; Gilchrist et al., 2010). Pausing offers a temporal opportunity for RNA capping and quality control, thereby ensuring the integrity of the transcript prior to the commitment to elongation (Blears, Svejstrup, 2025).

In addition, the paused state separates the rate of initiation from the rate of transcription. This enables the control of gene expression through the regulation of the duration of the paused state. This form of gene expression regulation is especially useful for genes that are needed during development or in response to environmental cues.

SUMMARY

Promoter-proximal pausing of Pol II results from the interplay between intrinsic early elongation instability and the active stabilisation provided by DSIF and NELF. DSIF alters the kinetic dynamics of elongation, whereas NELF stabilises a specific, typically transient backtracked state, collectively establishing a long-lasting yet reversible checkpoint. Pause release signifies a qualitative reconfiguration of the elongation complex facilitated by P-TEFb. Passing from descriptive genomic models to causal explanations requires an understanding of pausing at this mechanistic level, which is crucial for interpreting metazoan transcriptional control.

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RNA POLİMERAZA II-NİN PROMOTOR-PROKSİMAL PAUZASI: ERKƏN ELONQASİYANIN DSIF VƏ NELF VASİTƏSİLƏ TƏNZİMLƏNMƏSİNİN MOLEKULYAR MEXANİZMLƏRİ

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RNA polimeraza II-nin (Pol II) promotor-proksimal pauzası metazoylarda transkripsiyanın əsas tənzimləyici mərhələlərindən biri hesab olunur, lakin onun molekulyar mexanizmləri elmi ədəbiyyatda kifayət qədər dərindən araşdırılmamışdır. Mahiyyət etibarilə pauza, DSIF və NELF elonqasiya faktorlarının təsiri altında, həmçinin RNA polimeraza II-nin daxili xüsusiyyətləri, yeni sintez olunan RNA, DNT ardıcıllığı və xromatin arxitekturası ilə birlikdə erkən elonqasiya kompleksinin sabitləşdirilməsi prosesidir. Bu icmalda RNA polimeraza II pauzasının geniş mexanistik təhlili təqdim edilir və xüsusi olaraq DSIF və NELF faktorlarının elonqasiya kompleksini fiziki və kinetik baxımdan necə yenidən proqramlaşdıraraq sabit və geri dönən pauza kompleksi formalaşdırdığı, həmçinin bu kompleksin sərbəst buraxılmasının məhsuldar elonqasiyanın başlanğıcını müəyyən edən geri dönməz hadisə olduğu araşdırılır.

Xüsusilə promotoru tərk etdikdən sonrakı erkən elonqasiya mərhələsi təhlil olunur. Bu mərhələdə RNA polimeraza II yüksək konformasion dinamika nümayiş etdirir və pauzaya, geriyə sürüşməyə, abortiv transkripsiyaya və abortiv translokasiyaya meyilli olur. Bu isə elonqasiya kompleksinin kinetik balansını məhsuldar elonqasiya və ya pauza vəziyyəti istiqamətində dəyişə bilən tənzimləyici təsirlərə yüksək həssas mühit yaradır. Məqalədə DSIF-in həm RNA, həm də polimerazanın müxtəlif struktur elementləri ilə qarşılıqlı əlaqəyə girərək polimerazanın hərəkətini necə məhdudlaşdırdığı, bu prosesin isə NELF kompleksi tərəfindən necə əlavə sabitləşdirildiyi və nəticədə promotor-proksimal pauzanın formalaşdığı nəzərdən keçirilir. Bundan əlavə, fosforilləşmədən asılı remodelləşmə nəticəsində pauzada olan polimerazanın bu nəzarət mərhələsindən necə azad olduğu, eləcə də bu prosesin hüceyrədaxili siqnal yolları, xromatin vəziyyəti və inkişaf hadisələri ilə əlaqəsi araşdırılır. Təqdim olunan yanaşma promotor-proksimal pauzanı polimerazanın "emergent davranışı" kimi qiymətləndirməyə imkan verir; bu davranış elonqasiya faktorları tərəfindən aktiv şəkildə tənzimlənərək gen ekspressiyasının zaman və məkan baxımından dəqiq uyğunlaşdırılmasını təmin edir.

Açar sözlər: *Pol II pauzası, NELF, DSIF, pauzadan çıxış, P-TEFb*

ПРОКСИМАЛЬНАЯ ПАУЗА РНК-ПОЛИМЕРАЗЫ II У ПРОМОТОРА: МОЛЕКУЛЯРНЫЕ МЕХАНИЗМЫ КОНТРОЛЯ РАННЕЙ ЭЛОНГАЦИИ, ОПОСРЕДОВАННЫЕ DSIF И NELF

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Проксимальная пауза РНК-полимеразы II (Pol II) у промотора представляет собой ключевое регуляторное событие транскрипции у метазойных организмов, однако её молекулярные механизмы в научной литературе освещены недостаточно подробно. По своей сути пауза представляет собой стабилизацию специфического комплекса ранней элонгации под действием факторов элонгации DSIF и NELF в сочетании с внутренними свойствами РНК-полимеразы II, новосинтезированной РНК, последовательности ДНК и архитектуры хроматина. В данном обзоре

предпринята попытка представить всесторонний механистический анализ паузы РНК-полимеразы II с особым акцентом на то, каким образом DSIF и NELF физически и кинетически перепрограммируют комплекс элонгации, формируя стабильный и обратимый паузный комплекс, а также каким образом высвобождение этого комплекса становится необратимым событием, знаменующим начало продуктивной элонгации.

Особое внимание уделено ранней стадии элонгации после выхода полимеразы из промотора, в ходе которой РНК-полимераза II характеризуется высокой конформационной динамичностью и подвержена паузированию, обратному смещению, абортивной транскрипции и абортивной транслокации. Это создаёт условия, чрезвычайно чувствительные к регуляторным воздействиям, способным смещать кинетическое равновесие комплекса элонгации в сторону продуктивной элонгации либо формирования паузного состояния. Рассматривается, каким образом DSIF взаимодействует как с РНК, так и с различными структурными элементами полимеразы, ограничивая её продвижение, а также как данный процесс дополнительно стабилизируется комплексом NELF, обеспечивающим формирование проксимальной паузы у промотора. Кроме того, анализируется, как фосфорилирование-зависимое ремоделирование паузированной полимеразы устраняет данный контрольный этап, связывая высвобождение паузированных полимераз с клеточными сигнальными путями, состоянием хроматина и процессами развития. Представленный подход позволяет рассматривать проксимальную паузу у промотора как «эмерджентное свойство» полимеразы, активно регулируемое факторами элонгации и обеспечивающее тонкую пространственно-временную настройку экспрессии генов.

***Ключевые слова**: паузирование Pol II, NELF, DSIF, высвобождение из паузы, P-TEFb*

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