

## Nucleosomal Barrier to Transcription: Structural Determinants and Changes in Chromatin Structure

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### Abstract

Packaging of DNA into chromatin affects all processes on DNA. Nucleosomes present a strong barrier to transcription, raising important questions about the nature and the mechanisms of overcoming the barrier. Recently it was shown that DNA sequence, DNA–histone interactions and backtracking by RNA polymerase II (Pol II) all contribute to formation of the barrier. After partial uncoiling of nucleosomal DNA from the histone octamer by Pol II and backtracking of the enzyme, nucleosomal DNA recoils on the octamer, locking Pol II in the arrested state. Histone chaperones and transcription factors TFIIS, TFIIF and FACT facilitate transcription through chromatin using different molecular mechanisms.

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### Description

Transcribing RNA polymerase II (Pol II) induces extensive chromatin remodeling facilitated by histone chaperones and elongation factors and accompanied by limited histone exchange [1]. At the same time, histones are fully evicted only from highly transcribed genes [1]; thus Pol II typically encounters nucleosomes during transcription of every ~200 bp DNA regions. Nucleosomes remaining on transcribed genes form two types of barriers for transcribing Pol II [2,3]. In yeast and *Drosophila* each nucleosome presents a barrier where Pol II is paused after transcribing ~15 and ~50 bp from the nucleosome boundary [2,3]; these barriers are also universally observed *in vitro* [4]. A much higher barrier of the second type is formed when the active center of the enzyme is positioned ~10 bp upstream of the first (+1) transcribed nucleosome in *Drosophila* [3]. However, the relative contribution to this pause from the +1 nucleosome and negative elongation factors is not clear, particularly for highly expressed genes [3].

When Pol II encounters a barrier during transcript elongation, either DNA-bound proteins or DNA sequences that disfavor addition of the next NTP, polymerase backtracks by sliding the transcription bubble and RNA-DNA hybrid upstream along the template. This displaces the RNA 3' end from the Pol II active site, resulting in transcriptional arrest. Rapid relief of arrest requires protein factor TFIIS, which acts along with the Pol II active center to drive cleavage of the transcript. This restores alignment of the

3' end with the active center and releases the downstream RNA segment [5]. Arrest sites are rare within DNA but backtracking and arrest are general properties of Pol II complexes halted just downstream (~+17 to +32) of transcription start [6]. This is potentially important for the interaction of newly-initiated Pol II complexes with the +1 nucleosome.

A single nucleosome typically forms a high, asymmetrical barrier of the first type for Pol II transcription *in vitro* [4,7]; however, the putative regulatory -10 barrier of the second type observed *in vivo* has not been recapitulated *in vitro*. The strong +15 and +50 nucleosomal barriers are nucleosome-specific, Pol II-specific, and were described for all analysed organisms, from yeast to human

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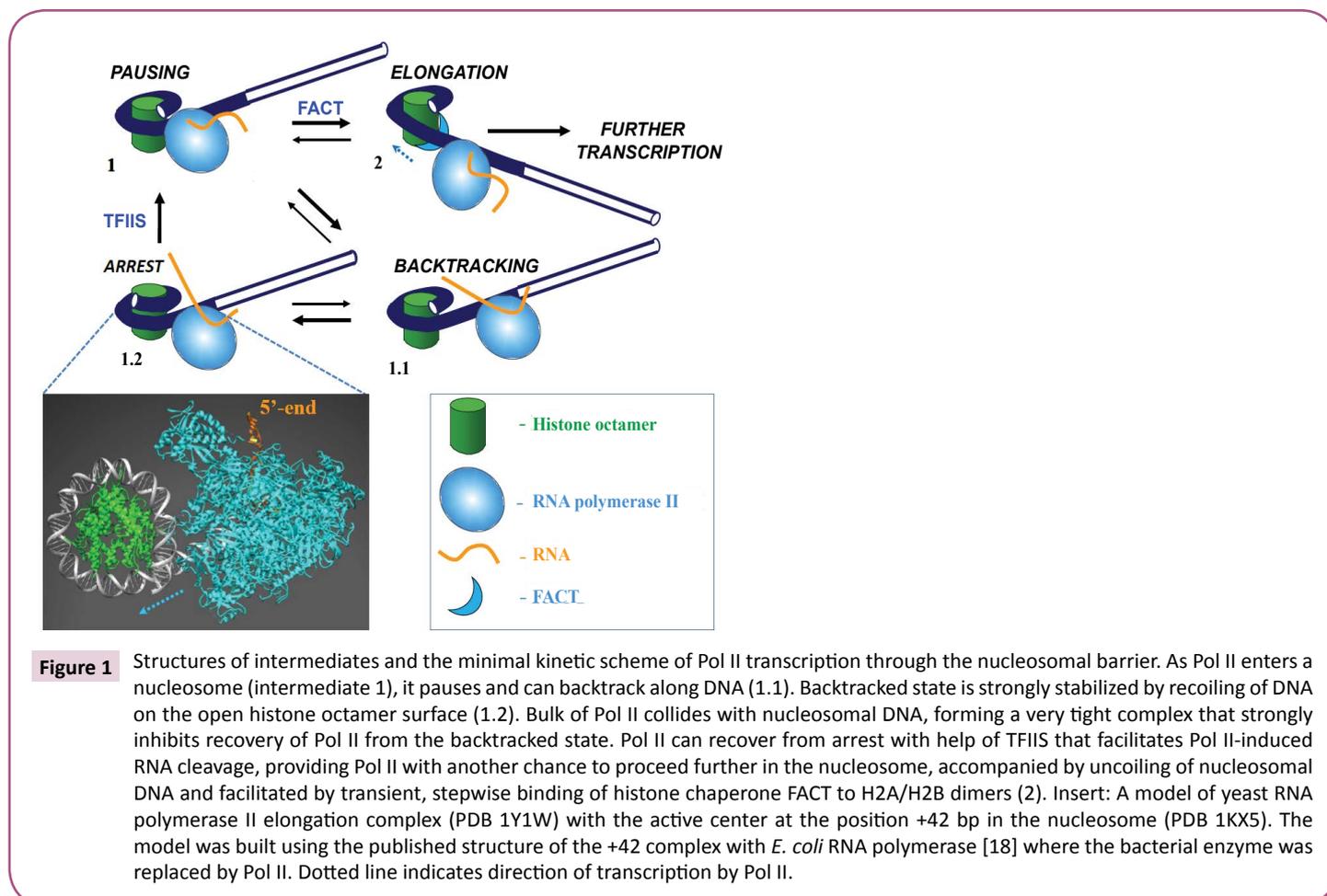
[4,8,9]. On any given DNA sequence wrapped onto a nucleosome, the barrier forms in distinct positions within the +(10-20) and +(40-50) regions [4]. Thus, these nucleosomal barriers are “universal signatures” of transcription through chromatin by Pol II both *in vitro* and *in vivo* [2-4].

Both DNA-histone interactions and Pol II pausing and backtracking contribute to formation of the barrier. Mapping of intranucleosomal DNA-histone interactions in single nucleosomes [10,11] identified two regions of strong interactions [(+25-35) and +(70-80)] that significantly affect the rate and efficiency of Pol II progression through a nucleosome, contributing to formation of the +15 and +50 nucleosomal barriers, respectively [4]. An additional DNA region +(89-102), the polar barrier sequence, determines overall affinity of DNA-histone interactions in a sequence-specific way, contributes to the +50 pausing and dictates overall height of the nucleosomal barrier to transcription [12,13]. The height of the barrier can also be strongly affected by a single nick in nucleosomal DNA [14].

The nucleosomal barrier is largely relieved after Pol II advances beyond position +49. Initially a small, Pol II-containing intranucleosomal DNA loop ( $\emptyset$ -loop) forms on the surface of the histone octamer at position +49 [12,15]. The  $\emptyset$ -loop is stabilized by Pol II-histone interactions that transiently and locally replace DNA-histone interactions [16]; the high efficiency of  $\emptyset$ -loop formation is characteristic for the Pol II-specific mechanism of

transcription through chromatin [17]. Formation of the  $\emptyset$ -loop induces uncoiling of the ~100-bp DNA region in front of the enzyme allowing further transcription through the nucleosome and efficient survival of nearly all histones (with exception of one H2A/H2B dimer that is displaced by Pol II) during this process [12,15]. The high efficiency of histone survival during transcription is explained in part by allosterically stabilized intranucleosomal histone-histone interactions [18]. Recent structural analysis indicates that after Pol II encounters the strong +50 barrier, the enzyme backtracks and nucleosomal DNA re-coils on the octamer, locking Pol II in the arrested state (**Figure 1**) [18].

Two general mechanisms should facilitate nucleosome traversal: holding Pol II in its active state, including facilitating recovery from arrest, and disrupting critical histone-DNA interactions. As noted, TFIS mediates transcript cleavage to restart arrested polymerases and facilitates transcription through chromatin *in vitro* [8,19,20] (**Figure 1**). In metazoans, TFIIF maintains Pol II's catalytic readiness and thus substantially increases overall elongation rates. Both TFIIF and TFIS are associated with the body of active genes [21]. *In vitro* studies showed that these two factors together modestly facilitate elongation through a single nucleosome. However, with a nucleosome containing a Sin mutant histone, which weakens the critical octamer-DNA interactions near the nucleosome dyad, elongation in the presence of TFIIF and TFIS nearly matched the efficiency and rate of elongation on histone-free DNA [22,23]. These *in vitro*



studies with a minimal transcription machinery demonstrate that efficient and rapid nucleosome traversal is clearly possible when Pol II is optimized and DNA unwrapping from the octamer is facilitated [24]. Histone chaperone FACT is an example of a factor that facilitates DNA unwrapping from H2A/H2B dimers to relieve the nucleosomal barrier and facilitate nucleosome traversal by Pol II [25,26] (**Figure 1**). Histone acetylation [27] and/or multiple molecules of Pol II [28,29] also help to overcome the barrier, affecting different steps during transcription through chromatin *in vitro*. While wrapping of DNA on the central core of the histone octamer provides the primary block to transcript elongation, *in vitro* studies using histones lacking the N-terminal tails showed that the tails also contribute to the nucleosomal barrier [27,30].

Future studies in this area should address more fully the mechanisms through which Pol II overcomes the two classes of nucleosome-induced pauses described above. It has been suggested that the nearly universal pause by metazoan Pol II at ~50 nt downstream of transcription start is directly linked to the barrier imposed by the +1 nucleosome [3], consistent with the general tendency of Pol II to backtrack early in elongation [6]. While pausing ~10 bp upstream of a promoter-proximal

nucleosome has not been observed *in vitro*, earlier studies did not incorporate known negative elongation factors, including NELF and DSIF (reviewed in [31]). Once Pol II has overcome promoter-proximal pausing, the polymerase will encounter barriers at ~15 and 50 bp within each downstream nucleosome [3]. Entry into productive elongation *in vivo* requires at least the activity of P-TEFb, but the full set of factors essential for pause relief and rapid long-range transcription has not been identified [31]. While proof of principle experiments with TFIIF and TFIIS have shown that the nucleosome is not an insurmountable barrier to elongation by Pol II [22], a major long term challenge will be to evaluate the roles of the much larger set of elongation-associate factors [31] in studies which require Pol II to rapidly and effectively traverse long arrays of nucleosomes *in vitro*.

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