

## ■ MOLECULAR BIOLOGY

**Nature of the Nucleosomal Barrier to RNA Polymerase II**

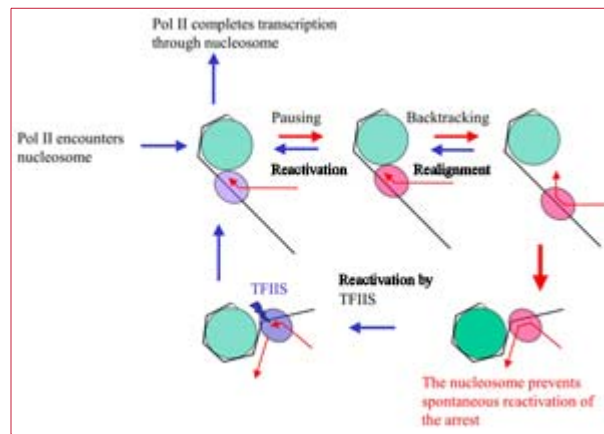
Kireeva ML, Hancock B, Cremona GH, Walter W, Studitsky VM, and Kashlev M. Nature of the nucleosomal barrier to RNA polymerase II. *Mol Cell* 18: 97–108, 2005.

Eukaryotes use the ordered packaging of template DNA into nucleosomes for regulation of gene expression. Examples of genes regulated at the level of transcription elongation in a nucleosome-dependent manner are *c-Fos* and *c-Myc* in mammals, *Hsp* genes in *Drosophila*, and *Met16* in yeast. In all these cases, the nucleosome positioned within the transcribed regions close to the promoter is required to establish an elongation checkpoint. When gene repression takes place, the elongation complex is stalled there. In response to an activation signal, RNA polymerase is rapidly released to start the elongation process. Establishing the sequence of events leading to chromatin-dependent changes in gene expression is an intriguing fundamental scientific problem and has important biomedical significance. Targeted regulation of the expression of oncogenes and tumor suppressors reveals new opportunities for the development of anticancer therapies.

Nucleosomes have long been considered powerful barriers to transcription elongation. This view is based on *in vitro* observations of a nucleosome-dependent inhibition of elongation by human RNA polymerase II (Izban MG and Luse DS. *Genes Dev* 5: 683–96, 1991). The observations suggest that *in vivo*, the nucleosomes must be modified so that the DNA-histone contacts are weakened enough to allow passage of the RNA polymerase (reviewed by Sims RJ 3rd et al. *Genes Dev* 18: 2437–68, 2004). However, despite multiple attempts, none of the nucleosome remodeling or modification events tested (including H2A/H2B dimer dissociation, or histone acetylation) brings the transcription rate on the chromatin template close to the physiological level. Our work challenges the hypothesized obligatory nature of chromatin remodeling and modification for efficient transcription elongation. We established that, in principle, the nucleosome does not present a specific barrier to RNA polymerase II. The observed transcription block is attributable to stabilization of intrinsic, sequence-specific pauses and arrests. Our findings emphasize the primary role of the elongation complex properties over the chromatin modification state for overcoming the nucleosome barrier by RNA polymerase II.

The first key observation we made was that transcription arrests induced by nucleosomes occur in the same sites as the sequence-dependent pauses on the nucleosome-free DNA. Notably, two nucleosomes, differently positioned on the same DNA sequence, caused the

same pattern of nucleosome-induced arrests. Furthermore, polymerase stalling at most of the positions within the nucleosomes leads to transcription arrest. Thus, interruption of the processive movement of RNA polymerase II on the nucleosomal template is necessary for the development of the nucleosome-specific transcription arrest and is the first step in this process (Figure 1).



**Figure 1.** Nucleosome-induced transcription arrest. The nucleosome is viewed from top. Only one superhelical turn of the DNA around a histone octamer (green circle) is shown. The RNA in the elongation complex is shown as a red arrow. RNA polymerase in the active elongation complex is shown in blue; in the paused or arrested elongation complex, it is shown in pink. Pol II, RNA polymerase II; TFIIS, transcription factor S-II.

Next, we examined the mechanism of arrest. On free DNA, arrest occurs by backtracking (Komissarova N and Kashlev M. *Proc Natl Acad Sci U S A* 94: 1755–60, 1997). Not surprisingly, blocking RNA polymerase II backtracking prevented nucleosome-induced arrest both in the stalled complexes and during continued transcription. Thus, we showed that nucleosome-induced arrest also occurs by the backtracking mechanism (Figure 1). Moreover, backtracking of RNA polymerase II leads to stabilization of the histone-DNA contacts in the nucleosome. From this we conclude that during processive RNA synthesis, the polymerase successfully disrupts DNA-histone contacts. However, when the elongation complex stalls and backtracks, the disrupted DNA-histone contacts are established again, preventing spontaneous reactivation of the elongation complex (Figure 1).

Once the polymerase is trapped in the inactive state by the DNA-histone contacts reestablished downstream of the backtracked elongation complex, the nucleosome indeed becomes a block to further elongation. The transcription resumes only in the presence of the anti-arrest factor transcription factor S-II (TFIIS). TFIIS promotes endonucleolytic cleavage of the RNA, which creates a new 3' end properly positioned in the active center. The reactivated polymerase acquires another chance to overcome the nucleosome. We observed that in the presence of TFIIS, the polymerase was more likely to transcribe through the nucleosome. However, even with TFIIS, the elongation remained slow, presumably because of the multiple transcript cleavage and re-synthesis cycles occurring in the presence of TFIIS. It is likely that the nucleosome-induced arrest rarely develops in the cell, because it is blocked at any of the three preceding stages (pausing, backtracking, and reestablishment of

the DNA-histone contacts downstream of the backtracked elongation complex, such that the nucleosome prevents spontaneous reactivation of the arrest).

In summary, we have uncovered the following sequence of events leading to nucleosome-induced transcription arrest:

- RNA polymerase II efficiently disrupts the DNA-histone contacts until it encounters an intrinsic pause site and stops there.
- The elongation complex pauses and then backtracks.
- The histone-DNA contacts, formed downstream of the polymerase, stabilize the inactive backtracked conformation of the elongation complex.

Notably, only the last step is nucleosome specific and may be dependent on chromatin remodeling and modification. The first two steps, as well as TFIIS-dependent reactivation, are nucleosome independent. Thus, transcription factors that change the efficiency of sequence-specific pausing and backtracking should be powerful regulators of transcription in the context of chromatin, along with chromatin remodeling and modification factors.

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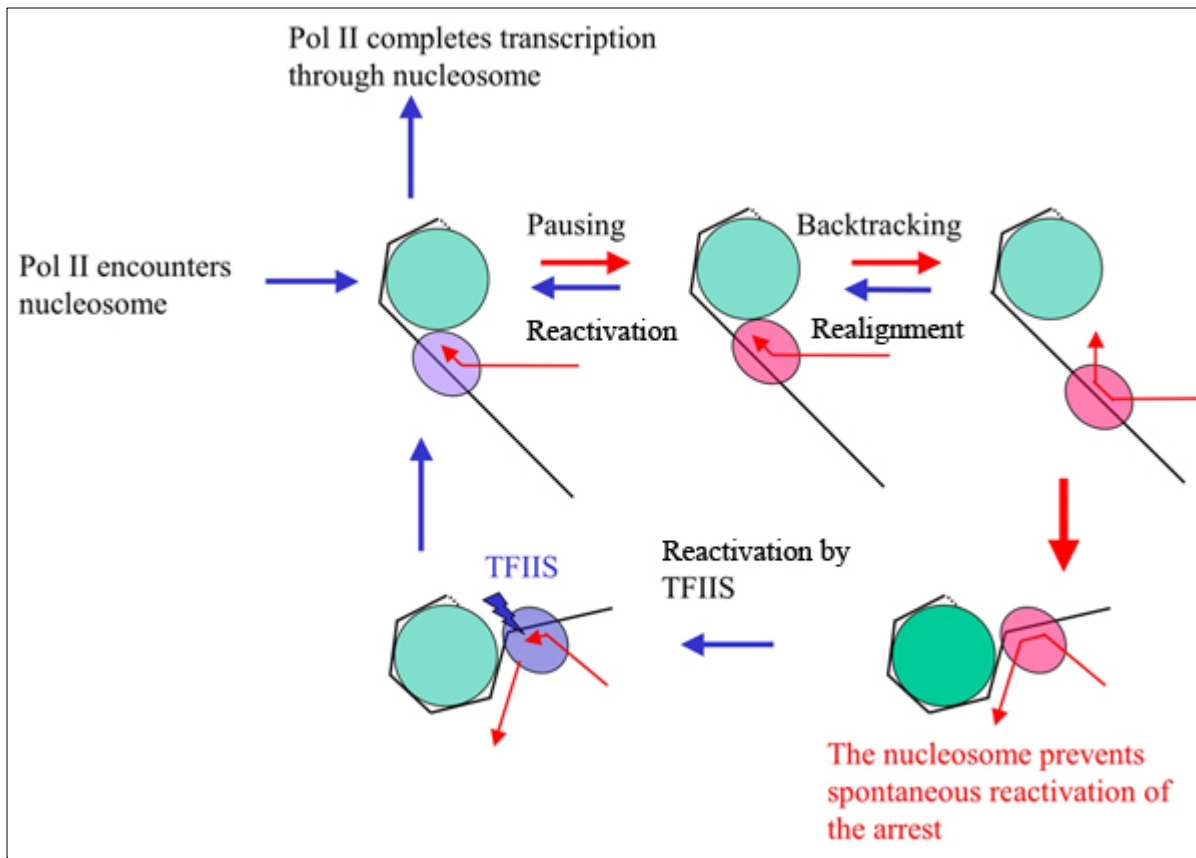
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