Molecular Cell, Vol. 9, 451–457, March, 2002, Copyright ©2002 by Cell Press

Previews

How Does Pol II Overcome the Nucleosome Barrier?

DNA packaging into chromatin presents a strong barrier to RNA polymerase II transcription. In this issue of *Molecular Cell*, Kireeva et al. describe a minimal system to examine polymerase II transcription through a positioned nucleosome and show, surprisingly, that transcription leads to the displacement of an H2A·H2B dimer from the nucleosome without altering nucleosome position.

The DNA within eukaryotic cells is tightly packed into chromatin. While this allows for control of gene regulation by constraining interaction with specific DNA sequences, it also serves as a major obstacle to protein factors that need access to DNA. Although chromatin structure is known to be decondensed in actively transcribed DNA, nucleosomes are still present throughout the transcribed region. The nucleosome contains 146 bp of DNA wrapped around a protein octamer core, which is composed of a central tetramer of two copies each of histones H3 and H4 flanked on either side by dimers of histones H2A and H2B. Polymerase (Pol) II must not only penetrate chromatin, but it must move processively through genes that can contain from several to several thousands of nucleosomes.

In vitro transcription assays have established that, at physiological salt concentrations, the nucleosome alone is sufficient to abrogate nearly all transcription by purified Pol II (Izban and Luse, 1992). Particular elongation factors such as TFIIF or TFIIS, which stimulate elongation on naked DNA templates, are unable to alleviate this block to transcription. These results led to a search for protein factors that would improve the efficiency of Pol II transcription through nucleosomes. Factors identified in this way include the SWI/SNF chromatin remodeling complex and FACT, which has been reported to interact with histones H2A·H2B (Orphanides and Reinberg, 2000 and references therein). However, the mechanism by which these or other factors facilitate transcription on chromatin templates has been difficult to establish, largely due to the lack of a reconstituted in vitro transcription system wherein one can assay both the efficiency of transcription and the fate of the nucleosome.

To address this mechanistic issue, researchers from the Kashlev and Studitsky groups have joined efforts to develop a minimal in vitro system for studying transcription through a single positioned nucleosome. The authors use a novel method of elongation complex formation that requires only highly purified Pol II. The preformed elongation complex is ligated to a segment of DNA containing a mononucleosome, whose position can be mapped both before and after transcription (see Figure).

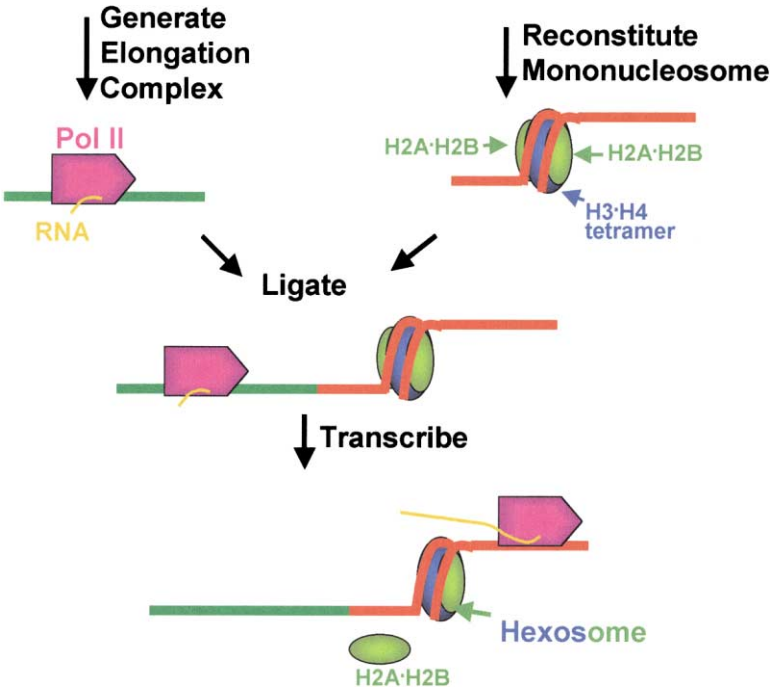
Kireeva et al. (2002) demonstrate that, while the nucleosome presents an almost insurmountable barrier to transcription by Pol II under physiological conditions, elevated salt concentrations permit a significant fraction of polymerases to overcome this obstacle. Surprisingly, the passage of Pol II through the nucleosome leads to nearly quantitative loss of one H2A·H2B dimer, leaving behind a hexameric nucleosome structure on the DNA that appears to be positioned at the same location as the initial octamer.

These results indicate that Pol II navigates through the nucleosomal barrier in a manner distinct from Polymerase III (Pol III) or the SP6 bacteriophage enzyme. These polymerases have been shown to pass an intact nucleosome upstream along DNA. Pol III and Sp6 are thought to achieve "octamer transfer" by forming DNA loops that are propagated through the nucleosome, permitting continuous reassociation of histones with upstream DNA (Studitsky et al., 1997). In contrast, Pol II does not displace the nucleosome, implying that it employs a different mechanism of accessing the nucleosomal DNA.

The role of the hexasome during transcription merits further evaluation. First, it has not yet been demonstrated in this system that formation of a hexasome facilitates transcription. Previous in vitro transcription assays on nucleosomal arrays have demonstrated that the H3·H4 tetramer poses as great an obstacle to transcription by Pol II as does the nucleosome (Chang and Luse, 1997). However, there might be something unique about the conformation of a hexasome that stimulates interaction of Pol II with nucleosomal DNA. Earlier studies indicated that nucleosomes depleted of one H2A·H2B dimer presented a significantly diminished barrier to transcription by Pol II (reviewed in van Holde et al., 1992). Thus, it would be interesting to see whether loss of an H2A·H2B dimer facilitates elongation through a mononucleosome or whether the improved elongation seen previously for dimer-depleted nucleosome arrays is an indirect consequence of diminished internucleosome interactions.

Second, although a hexasome results from transcription in this purified in vitro system, intact nucleosomes are found on active genes in vivo. While the level of in vivo crosslinking of the H2A·H2B globular histone domains to the *Hsp70* gene was diminished when this gene was activated to high transcriptional levels, crosslinking of histone tails to DNA within the highly transcribed gene showed no significant loss of any of the core histone proteins (Nacheva et al., 1989). These data suggest that, in vivo, the nucleosome core undergoes transient structural changes while the associated DNA is being transcribed. That these changes reflect the reversible displacement of an H2A·H2B dimer is in agreement with a large body of evidence suggesting increased exchange of H2A·H2B in regions of active transcription, with no overall depletion of any of the core histones.

Combining previous data with the current results suggests a model wherein Pol II transiently disrupts the octamer structure as it enters the nucleosome, extruding the globular domain of an H2A·H2B dimer from the



Schematic Highlighting Both the Key Features of the Experimental Strategy and the Main Result

Note that the illustration is not meant to distinguish which H2A·H2B dimer is displaced.

nucleosome core. While the high salt conditions used in the present assay would destabilize contacts between histone N-terminal tail domains and DNA, under physiological conditions these contacts persist. The transient structure might be stabilized by factors such as FACT associating with the H2A·H2B dimer, facilitating reassociation of the nucleosome after Pol II progresses beyond that point. The continuous cycles of disruption and reassociation that would occur with each passing polymerase could explain the depletion of stably bound H2A·H2B dimers in nucleosomes during highly activated transcription (van Holde et al., 1992).

Now that a minimal in vitro system has been established to study Pol II transcription through a nucleosome, a number of interesting experiments are possible. An oligonucleosome template can be substituted for the mononucleosome template used in these initial studies to evaluate the crosstalk of nucleosomes in arrays that more closely resemble native templates. Additionally, chromatin templates can be assembled with histones that have specific tail modifications producing the "histone codes" associated with active and inactive chromatin (Jenuwein and Allis, 2001). The effects of specific factors on both the efficiency of elongation through nucleosomes and on the composition and positions of the transcribed nucleosomes can also be examined. Elongation factors like FACT that interact with H2A·H2B are prime candidates, but so too are a growing list of factors that have been shown to influence transcription elongation (Orphanides and Reinberg, 2000 and references therein). In this regard, it is intriguing that mutations in yeast H4 in residues at the interface with H2A·H2B show genetic interactions with SWI/SNF genes as well as another class of transcription elongation factors called Spts (Santisteban et al., 1997). Thus, this novel in vitro system will provide an invaluable means of probing the mechanisms that act together in vivo to satisfy the extraordinary demands on Pol II processivity in its journeys through chromatin templates.

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Selected Reading

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