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Transcription through nucleosomes ☆

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Abstract

Transcriptionally active genes in eukaryotes still retain most of the Chromatin packaging that is characteristic of eukaryotic DNA. Nucleosomes and even some higher order structure are present, although the histones may be chemically modified, for example by acetylation or phosphorylation, as part of the activation process. The presence of nucleosomes on the coding region of active genes raises the question: How does an RNA polymerase transcribe such a template? We have attempted to answer this question with relatively simple model systems involving a template carrying a single positioned nucleosome. We have shown that when a phage polymerase, SP6, transcribes such a template, the histone octamer of the nucleosome is not released into solution. Instead it is retained on the same DNA molecule, but displaced from its original binding site. Further studies have allowed us to propose a detailed model, which appears to hold not only for SP6 but also for transcription by the much larger RNA polymerase III from yeast. Our most recent results, obtained by electron cryomicroscopy, confirm and refine this model. © 2000 Published by Elsevier Science B.V.

The DNA within the eukaryotic nucleus is packaged as chromatin, a well-defined complex composed of repeating subunits called nucleosomes. Each nucleosome consists of two left-handed superhelical turns of DNA, containing 165 base pairs, wound around a protein core of histones — an octamer of two each of histones H2A, H2B, H3 and H4. Strings of nucleosomes are further folded into chromatin fibers approximately 30 nm in diameter, and stabilized by the binding of linker histone H1 to the outside of the complex. Research in our own and other laboratories has shown that nucleosomes as well as some higher order structure are present even on

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actively transcribed genes. This raises the question: How can an RNA polymerase traverse a histone-covered template? During the past several years our laboratory has attempted to answer this question making use of simple model systems. The actual process in vivo is certainly much more complicated than that described here. There are protein complexes designed specifically to perturb nucleosome structure in a way that makes the histone octamers more mobile, and there is a wide variety of histone modifications that probably also serve that purpose. Nonetheless, as we show here, normal histone octamers have intrinsic acrobatic capabilities that can assist the passage of the polymerase.

Our earliest approach to this problem was suggested by the work of Liu and Wang [1], who observed that transcription of DNA templates under conditions where neither the template nor the transcription complex was free to rotate would result in accumulation of positive superhelical density in the DNA ahead of the polymerase, and negative supercoil behind. Since DNA is negatively supercoiled around the histone octamer in the nucleosome, it seemed possible that histones might be displaced as the polymerase advanced,

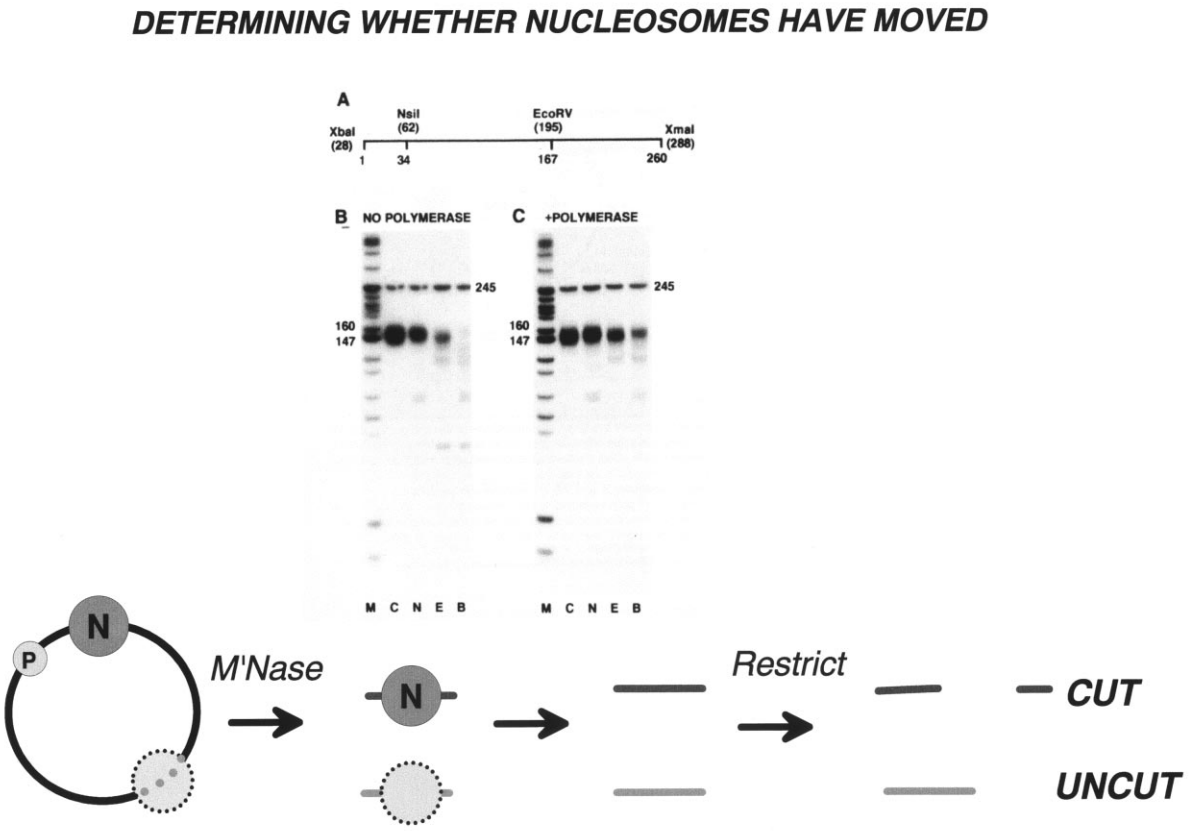

Fig. 1. (a) Restriction map of the 260-bp XbaI–XmaI fragment used to assemble the nucleosome core. It contains unique NsiI and EcoRV sites. All DNA from nucleosome cores (bottom, marked N; upper reaction) still at the original site after transcription will be sensitive to one or both of these enzymes. All DNA from cores at different sites (bottom, dotted circle) will be resistant to these enzymes. (b) Analysis of nucleosome core DNA from untranscribed circular templates after digestion with: N, NsiI; E, EcoRV; B, both enzymes; M, marker; C, no digestion. (c) Similar analysis after the template has been transcribed. (From Clark and Felsenfeld [4].)

and rebound behind it. We undertook a series of studies to measure the relative affinities of histone octamers under reversible conditions of binding [2]. Since binding of an octamer to a closed circular plasmid results in a positive contribution to the unconstrained superhelical density, binding to a positively supercoiled plasmid should be energetically less favorable than binding to a more negatively supercoiled plasmid. We found that this was the case, but also subsequently discovered that it is possible to form normal unperturbed nucleosomes even on positively supercoiled templates [3]. It therefore seemed unlikely that an advancing polymerase would perturb the structure of the octamer, though it might make it easier to displace it. The work described below makes it clear that in any case direct displacement into solution is not the mechanism by which polymerases advance on chromatin templates.

This early work led us to address directly the question: What happens to the histone octamer when a polymerase transcribes through it? We devised [4] a closed circular template (Fig. 1) carrying a single positioned nucleosome core situated downstream of a promoter for the viral RNA polymerase, SP6. In all cases described here the core histone octamer was used, and linker histones were omitted. In these structures approximately 147 bp of DNA are wound around the octamer. We also introduced transcription termination signals downstream of the nucleosome. The products of SP6 transcription were analyzed by making use of specific restriction sites within the templates, which were protected whenever they were covered by the histone octamer. We found that the template was readily transcribed. Analysis of the products showed that nucleosomes were not lost from the template, but were no longer present at their initial positions. This showed that the transcription mechanism did not result in displacement of the octamers in solution, nor had the octamers remained unmoved during passage of the polymerase, as had been suggested earlier (see references in [4]). Instead nucleosomes had been displaced to positions distributed over the entire plasmid, with some preference for regions behind the promoter. This led us to suggest that the octamers were either (1) displaced from the DNA but held in the electrostatic field of the DNA and then recaptured by the plasmid to which they had originally been bound, or (2) directly transferred out of the path of the advancing nucleosome to another site within the circle.

To distinguish between these possibilities we turned to linear templates only large enough to carry a single histone octamer [5]. When histones are reconstituted onto such a template (carrying an SP6 initiation site) they can occupy one of several distinct positions. These positional isomers can be distinguished by their mobilities in gel electrophoresis. It is then possible to determine their positions on the DNA by measuring the pattern of protection against digestion by restriction enzymes for existing sites within the template. We found that after transcription the oc-

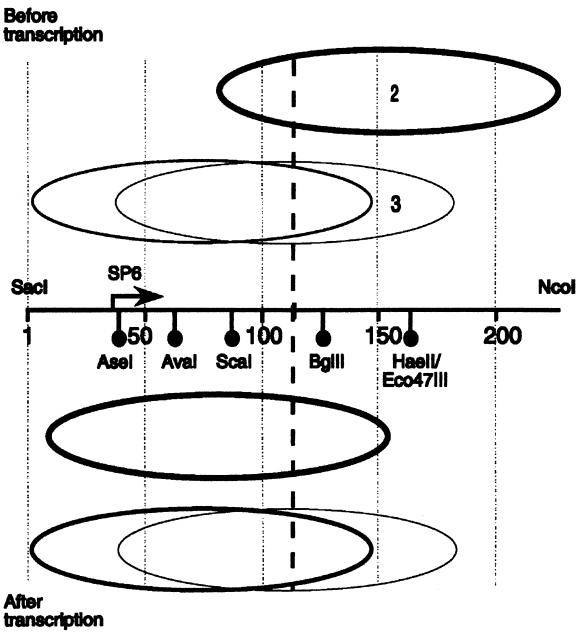

Fig. 2. Transcription on a 227-bp template carrying a single nucleosome core. Summary of nucleosome core positions before and after transcription. The cores drawn above the restriction map are those seen before transcription. Those below are positions observed after transcriptions. The complex labeled '2' represents the major starting position. The two positions marked in bold are the major product positions. Other positions marked by lighter lines are present as minor components. (From Studitsky et al. [5].)

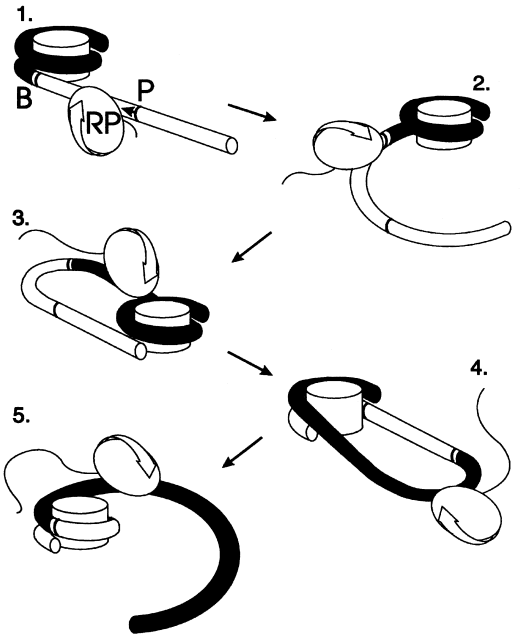

Fig. 3. First model: A spooling mechanism. (1) Polymerase binds to the DNA at the initiation site and (2) strips some DNA from the core surface as it advances. This eventually allows DNA at the 5′ end to rebind to the histone octamer surface and form a loop (3). The loop may be semi-stable, but in any event after the polymerase has advanced to some critical site (4), the 3′ end of the DNA pulls loose from the nucleosome core surface (5) and the polymerase is free to proceed to the end unimpeded. (From Studitsky et al. [5].)

tamer remained on its template, but had been displaced backward by 40–80 bp (Fig. 2). At moderate concentrations of nucleoside triphosphates (NTPs) transfer could be observed to cold competitor DNA at high competitor concentrations. At lower concentrations of NTPs there was little transfer to competitor. This result is consistent with the collision transfer model described above, but not with the release and recapture model. Further support for the former model comes from the observation that each positional isomer that undergoes transcription generates a corresponding distinct set of octamer positions after transcription. It is difficult to see how such 'memory' of the starting position could be retained if the octamer had been freed and recaptured.

These considerations led us to propose a mechanism for the transcription process. As shown in Fig. 3, we suppose that the polymerase enters the template and begins to strip DNA at the 5′ end of the template from the nucleosome surface. Eventually the freed DNA becomes of sufficient length that it can rebind to the octamer surface in such a way as to form a loop. By a process not yet specified, the polymerase can continue to advance until the amount of DNA ahead of the polymerase is sufficiently small so that the loop opens at the 3′ end. The polymerase is now free to complete transcription, because the octamer

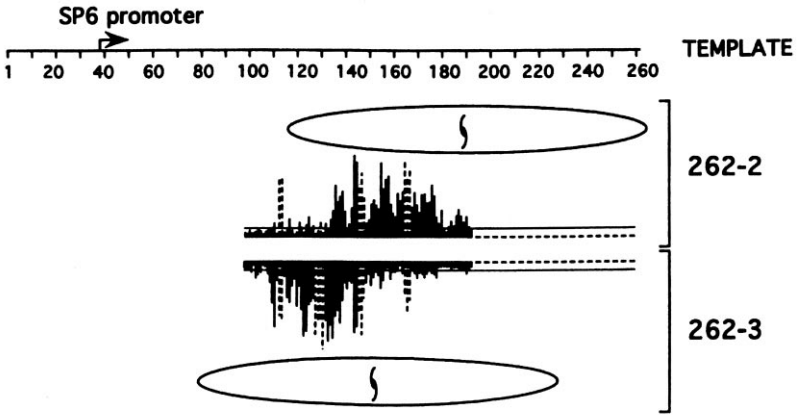

Fig. 4. Schematic diagram of pausing sites on nucleosome templates. The positions corresponding to pausing are shown as solid black bars proportional to band intensity. Dashed bars represent pausing sites on the naked DNA template. Results are shown for two templates with different positions of the octamer on the same DNA. (From Studitsky et al. [6].)

has transferred to a position behind it. This is of course why we observe the octamer moving backward on the template.

The critical point in this process occurs at the moment of transfer. To determine when this occurs we created a modified template carrying a C-less tract that allowed synchronization of transcription [6], since the polymerase could only advance a short distance before being arrested. We introduced a pulse label during initiation that allowed us to follow the RNA elongation process once the block to transcription was removed. As shown in Fig. 4, distinct pausing events could be seen as transcription proceeded. These were confined to the region of the template upstream of the nucleosome dyad axis. Beyond that point no further pausing was observed. This correlation with the dyad position was confirmed by transcribing a positional isomer that carried the same DNA, but with a different starting position of the octamer (Fig. 4).

These results demonstrate that the moment of octamer transfer occurs when the polymerase reaches the dyad axis. Before the transfer the octamer induces pausing of transcription; afterward there is no further impediment to the polymerase and therefore no pausing. One further question can be asked: Why does pausing occur? The simplest explanation would be that the bound octamer blocked advancement of the polymerase. More complex models arise if the formation of the transfer intermediate somehow slowed or accelerated transcription. To distinguish among these models we modified the template [6] so that after initiation and arrest of the polymerase the DNA 'tail' behind it could be shortened by digestion with one of three restriction enzymes. If the simple model is correct, this should make no difference. However, the experimental results (Fig. 5) show that shortening the tail has the effect of moving inward the point at which pausing commences. This shows that loop formation is important for pausing.

Quite recently, in collaboration with the laboratory of Dr Christopher Woodcock, we have extended these results by use of electron cryo-microscopy [7]. The templates were modified to extend the arrest point further into the nucleosome. It was then possible to observe paused intermediates in the microscope and correlate them with

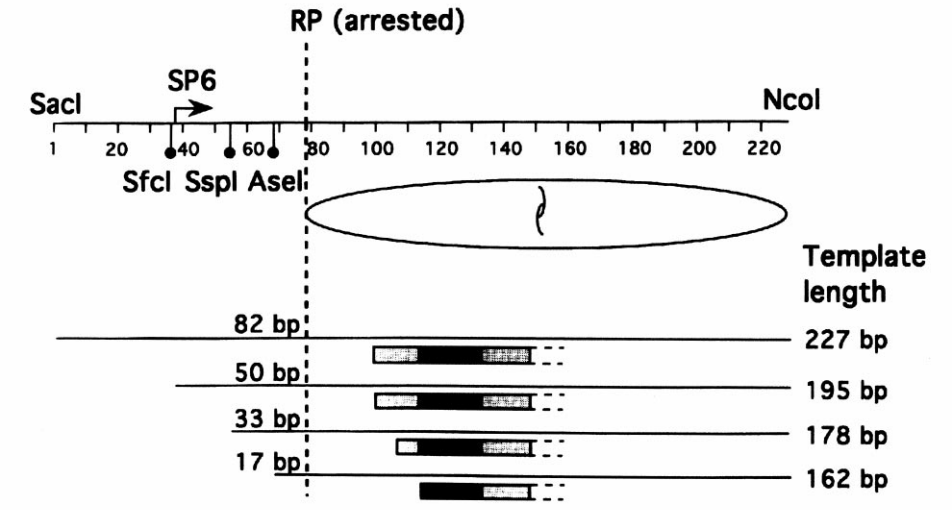

Fig. 5. Nucleosome-induced pausing on templates shortened by truncation of tails after loading of polymerase but before chain elongation. Regions of nucleosome-induced pausing are shown by stippled rectangles. Template sizes and lengths of DNA remaining behind the polymerase after restriction are shown. (From Studitsky et al. [6].)

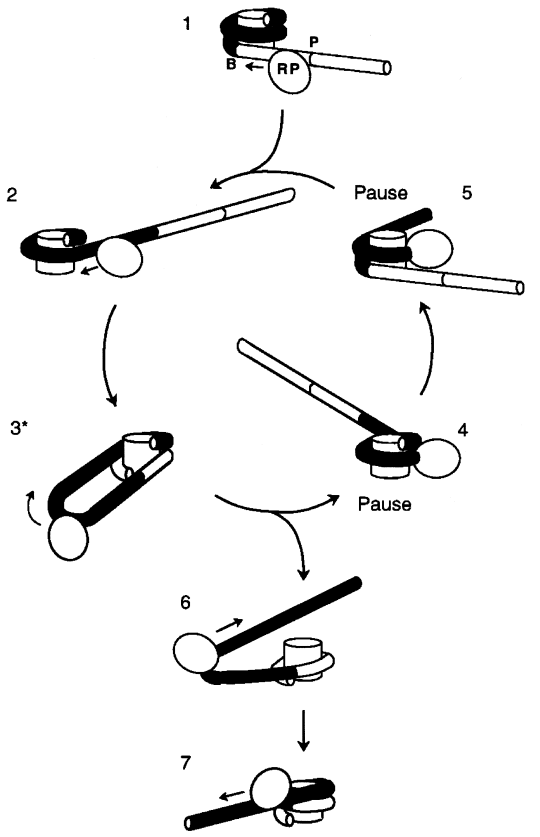

Fig. 6. A refined version of the original model for transcription through a nucleosome. (1) After RNA polymerase (RP) initiates transcription at the promoter (P) on a 227-bp template, it rapidly transcribes the first ~25-bp of nucleosomal DNA (shown in dark contrast, boundary B), causing partial dissociation of DNA from the octamer (2). The DNA behind the RP binds transiently to the exposed surface of the octamer forming a loop (3), which can be stabilized by superhelical stress. This can lead to collapse of the loop and formation of a one-tailed intermediate (CTI-1) where the RP is in close proximity to the histone core (4). The RNA polymerase now interferes with the NcoI end of the DNA, eventually causing its displacement and the formation of a two-tailed paused transcriptional complex, CTI-II (5), in which the further movement of RP is inhibited due to direct contact of the polymerase with the histone octamer. The dynamic equilibrium of the system, together with the force exerted by the polymerase, results in its release from the octamer, and the OTI configuration (2) is restored. This cycle of events may be repeated several times (not shown), as the polymerase progresses in ~10-bp increments. Eventually, when the polymerase has penetrated ~60 bp into the core, the tendency of the downstream portion of DNA to dissociate increases, giving rise to the conformation shown in (6) and completing the transfer of the octamer. Transcription continues freely (7) to the end of the template. (From Bednar et al. [7].)

biochemical analysis of the same samples. Most notably, we found some intermediates that we interpreted as arising from collapsed loops in which the polymerase is seen in close proximity to the octamer. We suggested that these are in fact the intermediate structures responsible for pausing, and that they can open to regenerate the loop and continue the transcription process. A summary of these conclusions is shown in Fig. 6.

All of these results were obtained with a prokaryotic polymerase, SP6. They nonetheless reveal intrinsic properties of histone octamers that should hold for all polymerases. To support this idea we have also turned to similar experiments with yeast RNA polymerase III [8]. These were carried out in collaboration with the laboratory of Dr E. Peter Geiduschek. The results obtained with this much larger enzyme were quite similar to those we had obtained with SP6 polymerase. The same internal octamer transfer and pausing behavior were observed.

We conclude that this mechanism of dealing with advancing polymerases is likely to be at work during eukaryote transcription. This is not to say that histone modifications [9] such as acetylation, or the action of nucleosome remodeling complexes such as Swi/Snf, will not play a role in the process of making templates more accessible. However, direct transfer of octamers is likely to be an important mechanism for allowing a polymerase to negotiate histone-covered templates. Our model system was designed to give the octamer limited choices so that transfer could be monitored, but on longer templates the octamer could be moved to any available vacant DNA site — even on another DNA molecule. We suggest that this may be the way in which all molecules that move along DNA, including RNA polymerases, DNA polymerases, and helicases, overcome the obstacles of chromatin packaging.

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