Hypothesis

A topological model for chromatin transcription and a role for nucleosome linkers

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There is a good deal of evidence that transcribing RNA polymerase may translocate across nucleosomes without their displacement and (or) rearrangement. A topological model for RNA chain elongation on a nucleosome is considered here. A new mechanism of RNA polymerase translocation is suggested in order to avoid the steric hindrances inherent in the model. It is shown that a transcribed nucleoprotein fiber should be interrupted by protein-free DNA stretches (nucleosome linkers) to allow release of nascent RNA. Possible verificiations and consequences of the model are discussed.

Chromatin *Nucleosome* *Transcription* *RNA polymerase* *DNA topology* *Supranucleosomal organization*

1. INTRODUCTION

The general features of eukaryotic chromatin organization as a linear array of repeating units, the nucleosomes, have been established in [1,2] and the mechanism of nucleosome dynamic perturbations, especially of chromatin transcription, is now waiting to be elucidated.

As a result of recent studies (review [3,4]) it has become clear that transcriptionally active non-ribosomal genes maintain their nucleosomal organization which is not disturbed in the vicinity of RNA polymerase [5] and even the higher-order structure may be retained in the transcribed chromatin portion [6]. The reaction of in vitro transcription on chromatin templates is not coupled with nucleosome dissociation [7–10] or with nucleosome sliding before the enzyme [10,11]. All these facts render very probable that both the eukaryotic and prokaryotic RNA polymerases can translocate across nucleosomes without their dissociation and rearrangement.

According to existing models based on studies of prokaryotic RNA polymerases the template-dependent RNA synthesis occurs via transient formation of hybrids between the growing 3′-end of RNA and the coding strand of DNA [12]. The DNA double helix is transiently melted at the point of RNA chain growth and the melted region is translocated during RNA elongation [13]. Thus, RNA polymerase is believed to take a helical path along the template. The application of this concept to models for chromatin transcription is faced with the problem of DNA shielding by histones. It has already led to the appearance of several models for nucleosome dynamic transitions in the course of RNA polymerase bypassing [14–17].

In this article, we argue that the mechanism of nucleosome transcription is based not on some unknown properties of nucleosomes but on the proposal that RNA polymerase has the ability to translocate on the nucleosome surface. The model would be compatible also with the possibility of transcription on supranucleosomal structures if one takes into account that the nucleosome fiber is interrupted by protein-free DNA stretches (nucleosome linkers).

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2. RELATIONSHIP TO OTHER WORK

Since the discovery that RNA polymerase may transcribe across nucleosomes several models have appeared for nucleosome transitions in the course of transcription. They may be summarized as follows [10,18]:

(i) Nucleosomes are structurally rearranged to allow unwinding of the DNA and histone;
(ii) Nucleosomes dissociate transiently at the moment of the enzyme bypassing; and
(iii) The basic nucleosome structure remains intact and only minor changes of DNA–histone bonds may occur.

The model for nucleosome unfolding in the course of transcription was originally proposed by Weintraub et al. [14] who stated that a nucleosome may dissociate along its dyad axis while the unpaired histones remain attached to the DNA strands and do not interfere with RNA polymerase. However, examination of this model has shown no specific effect of histone crosslinking on transcription [7,19]. This result is in obvious contradiction with Weintraub's model as the histone crosslinking is supposed to prevent unfolding of the nucleosome.

The mechanisms of transient nucleosome dissociation during movement of RNA polymerase were considered in several works. For instance it has been suggested that the histone octamer may be displaced to another core [15], to nearby available DNA [16] or to the non-coding DNA strand [17]. To date, no sliding or dissociation of nucleosomes during transcription has been found [9–11,20]. The enhanced nucleosome mobility in the in vitro transcription assay in [7] is not unequivocal evidence since the reaction conditions were very close to those promoting autonomous histone sliding which may be facilitated by RNA polymerase. Furthermore, histones have not been found to dissociate from a single transcribed nucleosome [8] and the ternary transcription complex was shown to be organized into nucleosome structures [5]. These facts do not exclude the existence of some very rapid and reversible histone dissociation but make this possibility rather dubious. It is also very difficult to explain on the basis of accepted models for nucleosome dissociation how the artificially reconstituted nucleosomes manage to jump over the passing enzyme and to stay at or near their initial place [10]. The distances between the reconstituted nucleosomes are too long and irregular to allow their interactions while the absence of nucleosome redistribution has been shown by studying histone transfer to competitor DNA [10]. The model of uncoiling only the coding strand from the nucleosome also does not fit since it is derived from an in vitro assay via the prokaryotic enzyme which is known to melt a DNA stretch of about 20 basepairs which is much less than nucleosome DNA [13].

We have not yet found any objections to the possibility of RNA polymerase translocation across structurally and positionally intact nucleosomes except that this mechanism is not compatible with the accepted model for RNA polymerase translocation. Here we show that the problem may be successfully circumvented if the real path of the elongated RNA chain is not exactly helical as it is supposed to be.

3. MODEL CONSIDERATIONS

An alternative mechanism of RNA polymerase translocation is based on the assumptions that the 3′-end of the growing RNA chain can be transiently displaced from its template without the dissociation of the whole transcription complex and that the interfering turns of the DNA double helix can be relaxed through this disconnection (fig.1). The achievement of the first of these two requirements is demonstrated by the facts that the DNA·RNA hybrid region in the ternary complex is too short to prevent the reiterative sliding of RNA polymerase on its template [12,21] and that the enzyme is capable of transcribing on discontinuous templates [22,23]. The second assumption remains hypo-

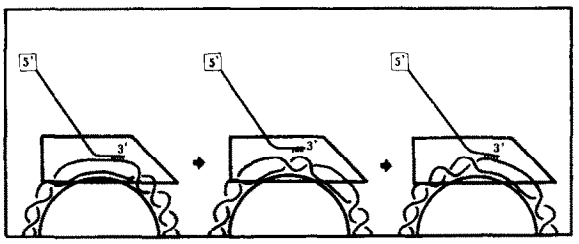

Fig.1. The sequence of steps in the non-helical translocation of RNA polymerase on a nucleosome surface.

thetical, although it is by no means incompatible with the known properties of both the prokaryotic and eukaryotic enzymes [12,24]. The model permits the RNA polymerase not to follow the helical path around the template and thus does not impose any spatial hindrance on the RNA polymerase translocation across a nucleosome.

An additional spatial problem may arise from the RNA winding around the template. The nascent RNA is known to be attached to nuclear matrix [25] and to ribonucleoprotein particles [26] in eukaryotic cell nuclei and RNA release by rotation of its 5′-end around the template therefore seems to be impossible. RNA unwinding by DNA rotation is also hindered owing to the presence of nucleosomes and supranucleosome structures.

According to the proposed model the nonhelical translocation of RNA polymerase should not lead to the appearance of any constraints on the releasing RNA. However, we have so far neglected the real DNA coiling. The existence of 1 or 2 left superhelical turns per nucleosome [27] would lead to the additional winding of nascent RNA around the nucleosome dyad axis. These RNA turns can be successfully released from a free superhelix or from a single nucleosome particularly because the sign of the superturns is opposite to that of DNA double helix. However, the higher order nucleosome organization of transcribed chromatin [6] makes the model a bit more complicated.

Consider a transcription event on a chain of stacked nucleosomes (fig.2a). Here the RNA turns around the nucleosome axis cannot be unwound without rotation of the whole nucleosome fiber. Separation of adjacent nucleosomes would help to overcome this problem since the mode of RNA unwinding on a chain of separated nucleosomes is the same as that on a free superhelix (fig.2b). Thus the significance of deoxynucleoprotein fiber interruption with protein-free nucleosome linkers may be deduced from the model.

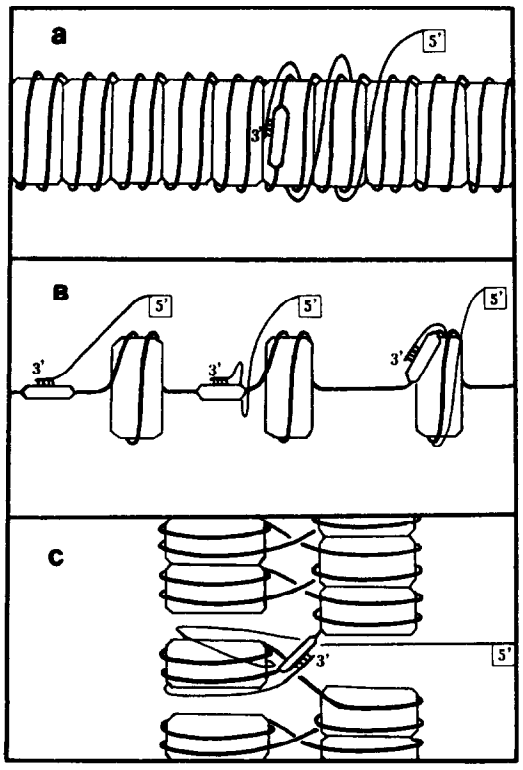

Fig.2. A topological model for chromatin transcription: (a) fiber of stacked nucleosomes; (b) beaded nucleosome chain; (c) zig-zag ribbon.

4. FEASIBLE VERIFICATIONS AND DISCUSSION

The model presented here states that RNA polymerase may transcribe across structurally intact nucleosomes. A set of experiments is consistent with this idea (see discussion above) though another explanation that the nucleosomes unfold under standard conditions but dissociation of histone cores occurs when the latter are crosslinked is not excluded. Simultaneous testing of both the possibilities is needed to examine the nucleosome transcription mechanism.

One may also suppose that the RNA polymerase bypasses a nucleosome via the partial dissociation of nucleosome DNA (e.g., of half of the molecule). Fixation of DNA coiling on the nucleosome surface should rule out this possibility while our model predicts that the transcription would not be halted if the DNA is immobilized by regions remote from the ternary complex. By attaching the ends of a nucleosome DNA molecule to a crosslinked histone octamer one may freeze the nucleosome topology in order to test the proposed model.

Several models for nucleosome stacking have been proposed to represent the structure of the 250–300 Å chromatin fiber [28–30] which is main-

tained through the gene activation [6]. The zig-zag ribbon of Worcel et al. [30] is in better agreement with our model than the other structures as it allows dissociation of the nucleosome without considerable rearrangement of the whole fiber (fig.2c).

According to our model separation of adjacent nucleosomes is needed to activate the 250–300 Å fiber. The recent finding that the nucleosome linker organization is altered in active ovalbumin genes [31] seems to support the idea. Active chromatin is associated with high mobility group proteins, HMG [4,32], whose role remains obscure. HMGs may interact with basic histone regions [32,33] while the latter are known to support nucleosome folding by screening the DNA negative charges [34,35]. Therefore it may be reasonable to suppose that HMG proteins facilitate the dynamic opening of nucleosome linkers by weakening the internucleosomal interactions.

The model for non-helical translocation has been considered here for both eukaryotic and prokaryotic RNA polymerases. Prokaryotes have no nucleosomes but their chromosomes are associated with proteins which may also require the non-helical translocation of the RNA polymerase. The non-helical mechanism may be also important for nascent RNA release which is spatially hindered in the prokaryotic cell where the nascent RNA is attached to polyribosomes and the template is supercoiled and thus not free to rotate. We hope that this hypothesis will promote new attempts to study the mechanism of RNA polymerase translocation.

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