Cell, Vol. 76, 371–382, January 28, 1994, Copyright © 1994 by Cell Press

A Histone Octamer Can Step around a Transcribing Polymerase without Leaving the Template

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Summary

The mechanism by which nucleosome cores are displaced and re-formed during transcription in vitro has been investigated. A nucleosome core was assembled on a short linear DNA template (227 bp) containing an SP6 RNA polymerase promoter and a nucleosome-positioning sequence. Transcription induced the translocation of the nucleosome core over 75 or 80 bp to two positions at the other end of the template, blocking the promoter. At low rNTP concentrations, transfer occurred only on the same template molecule, even in the presence of large excesses of competitor DNA. On a longer template (262 bp), nucleosome core position after transcription depended on its position before transcription. The data suggest that the octamer transfers without dissociation from DNA and provide strong evidence for a translocation mechanism in which DNA ahead of the polymerase uncoils from the octamer as the DNA behind coils around it. In this way, the octamer steps around the transcribing polymerase.

Introduction

Eukaryotic DNA is packaged into chromatin, coiled filaments of nucleosomes that resemble beads on a string when decondensed (Thoma et al., 1979). The nucleosome contains a core (the bead), a molecule of histone H1, and linker DNA (the string) that connects one core to the next. The core of the nucleosome is composed of ~146 bp of DNA wrapped in 1.75 turns around a central histone octamer containing two molecules each of the four core histones: H3, H4, H2A, and H2B (Richmond et al., 1984). A molecule of H1 seals the closely juxtaposed DNA turns in the core and also binds to the linker.

Transcriptionally active genes are partially depleted of H1 but retain a nucleosomal conformation (McKnight et al., 1978; de Bernardin et al., 1986; Björkroth et al., 1988; Nacheva et al., 1989; Kamakaka and Thomas, 1990; Bresnick et al., 1992). How does RNA polymerase negotiate a gene assembled into nucleosomes? Transcription through a highly compact nucleosome core by a large RNA polymerase molecule would appear to be sterically impossible. To account for this difficulty, two classes of models have been proposed (see reviews by the following: Thoma, 1991; Kornberg and Lorch, 1991, 1992; Felsenfeld, 1992; van Holde et al., 1992; Morse, 1992; Adams and Workman, 1993): first, unfolding of the nucleosome core through disruption of histone–histone interactions within the octamer such that histone–DNA contacts are preserved but the DNA coils are removed. This process would leave the position of the core unchanged. The second model is displacement of the nucleosome core by the polymerase (which would remove the steric problem altogether).

Recently, we presented evidence in favor of nucleosome core displacement and re-formation as a consequence of transcription (Clark and Felsenfeld, 1992). We assembled a nucleosome core on a short DNA fragment and ligated it into a plasmid containing a promoter for SP6 RNA polymerase to obtain a template with a core at a known site. Transcription induced the translocation of the core to other sites on the plasmid, with a moderate preference for the ~900 bp region of the plasmid behind the promoter. We concluded that the nucleosome core was displaced and re-formed elsewhere as a result of transcription, but we were unable to distinguish between a direct transfer mechanism involving a bridging intermediate in which the histone octamer interacts with both donor and acceptor DNA and an indirect transfer (release/recapture) mechanism involving a free histone intermediate that is almost immediately recaptured. In this paper, we distinguish between these two mechanisms using a very short DNA fragment containing an SP6 promoter linked to a nucleosome-positioning sequence as a template for transcription. We show that the histone octamer can step around the transcribing polymerase over a distance of only 40–95 bp, without leaving the template.

Results

Positioned Nucleosome Cores and Hexamer Complexes on a Short DNA Fragment

The template used for transcription was a 227 bp SacI–NcoI fragment containing a nucleosome-positioning sequence and an SP6 promoter (Figure 1A). The transcription start site is located 38 bp from the SacI end, and the runoff transcript is 189 nt long. A nucleosome core was reconstituted on the 227 bp template by salt and urea dialysis and separated from remaining free template in a sucrose density gradient. Unlabeled reconstitutes (for micrococcal nuclease digestion) and reconstitutes containing template labeled at the NcoI end (for nucleoprotein gel analysis) were prepared in parallel. Two fractions were selected from each gradient for study. Analysis of the histone contents of both fractions in an SDS gel revealed that the faster-sedimenting fraction contained all four core histones in equal amounts, but the slower-sedimenting fraction was deficient in H2A and H2B (data not shown). This suggested that the fast- and slow-sedimenting fractions contained histone octamers and hexamers (one H2A/H2B dimer missing), respectively. This was confirmed by chemical cross-linking of the histones in the reconstitutes using dimethyl suberimidate (data not shown), as described by Stein et al. (1977). The octamer fraction

Figure 1. Transcription Induces Translocation of a Nucleosome Core

(A) Restriction map of the 227 bp SacI–NcoI template. The template was labeled at the NcoI end. The cleavage sites for AvaI, ScaI, and HaeII are at positions 57, 83, and 162, respectively.

(B) Analysis of nucleosome cores in a nucleoprotein gel using the restriction enzyme protection assay. Complexes 1, 2, and 3 are indicated. Lanes 1 and 5: cores before transcription. Lanes 2–4 and 6–10: cores were incubated for 20 min in the presence or absence of polymerase and then digested with HaeII, ScaI, or AvaI, as indicated. Note: the template was labeled at the NcoI end; fragments having only the SacI end are not visible. M, end-labeled MspI digest of pBR322.

(C) Analysis of hexamer complexes in a nucleoprotein gel using the restriction enzyme protection assay. The hexamer complex (complex 1) is indicated. Lanes 1 and 5: hexamer complex fraction. Lanes 2–4 and 6–10: restriction digestion of hexamer complexes as in (B).

will be referred to below as nucleosome cores and the hexamer fraction as hexamer complexes.

Analysis of the nucleosome core (Figure 1B, lane 1) and hexamer complex (Figure 1C, lane 1) fractions in a nucleoprotein gel revealed several complexes. The nucleosome core fraction contained a quantitatively major complex (2), two minor complexes (1 and 3), and a small amount of free DNA (10% of total DNA). Complex 1 had the same mobility as the hexamer complex. The hexamer fraction contained a single hexamer complex but was 70% free template. The mobility of a nucleosome core in a nucleoprotein gel is determined by its position relative to the ends of the fragment (Meersseman et al., 1992). A nucleosome core positioned at the center of the fragment has the lowest mobility, and a core positioned at either end of the fragment has the highest mobility. All pairs of cores symmetrically positioned relative to the center of the fragment will have the same mobility. Thus, nucleosome cores can be resolved in the gel according to their positions, but a single band might contain two different cores at symmetrically related positions. Micrococcal nuclease was used to identify nucleosome core positions (see below); the results support this rule relating mobility to position. Complex 2 is a mixture of nucleosome cores at one of two positions, one on the positioning sequence (accounting for 70% of the cores) and another at a symmetrically related position over the promoter. Complex 3 is a nucleosome core formed at a central position on the template.

Transcription-Induced Translocation of a Histone Octamer on a Short Template

The effect of transcription on the stability of the nucleosome core was examined in a nucleoprotein gel (Figure 1B). To ensure that most of the templates were transcribed, sufficient polymerase was added to saturate the template (under these conditions, the transcription rate was linear with respect to template concentration; data not shown). Transcription resulted in a 10% increase in free template; the displaced octamers were probably transferred to other nucleosome cores (see below; some very faint bands of much lower mobility were observed). However, most of the nucleosome cores were apparently stable to transcription (Figure 1B, lanes 1 and 3). The same result was obtained for the hexamer complex (Figure 1C, lanes 1 and 3). Therefore, either these nucleosome cores were not transcribed, or they remained in place after transcription, or they were displaced and re-formed on the template.

A restriction enzyme protection assay (Morse, 1989) was used to discriminate among these possibilities. If a restriction site is located within a nucleosome core, it is protected from digestion by the enzyme. Restriction enzyme was added directly to the incubation before or after transcription in quantities sufficient for complete digestion of the nucleosome-free template in the sample. HaeII was used as a probe for the position of the nucleosome core before and after transcription; the site for this enzyme is near the center of the major core (Figure 1A). Before transcription, 70% of the nucleosome cores in complex 2 were resistant to HaeII (Figure 1B, lane 2); these are cores formed on the positioning sequence. The 30% of cores sensitive to HaeII are at the symmetrically related position over the promoter. The free template was cleaved to yield a fragment of the expected size. After transcription, 90% of the cores were sensitive to HaeII (Figure 1B, lane 4). The 10% of complex 2 still resistant to HaeII after transcription might represent untranscribed templates. Thus, nucleosomal templates resistant to HaeII before transcription are sensitive to HaeII after transcription.

Exposure of the HaeII site after transcription could be

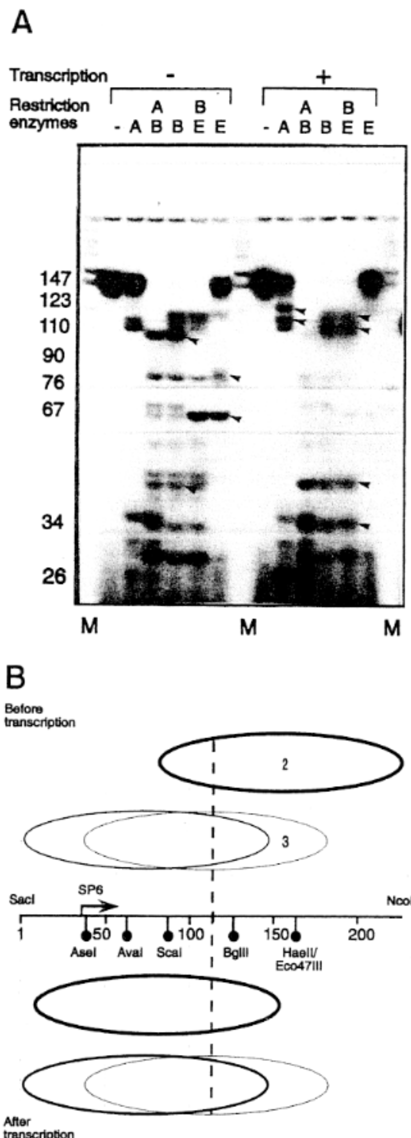

Figure 2. Positions of Nucleosome Cores on the 227 bp Template before and after Transcription

(A) Mapping of nucleosome core positions by restriction digestion analysis of purified core DNA. After incubation of nucleosomal templates for 20 min in the presence or absence of polymerase, core particles were prepared using micrococcal nuclease. Core DNA (145–155 bp) was gel-purified and labeled at both ends using T4 polynucleotide kinase, digested with various restriction enzymes (as indicated), and the products were analyzed in an 8% polyacrylamide gel. A, AseI; B, BglII; E, Eco47III. A 245 bp labeled control fragment was included in the digests to correct for losses (Clark and Felsenfeld, 1992). The discrete fragments produced on digestion of core DNA indicate the presence of positioned nucleosome cores; these were mapped by measuring the lengths of the bands and using double digests to determine the orientation of fragments with respect to restriction sites. Some cores were positioned so close together (<5 bp) that they were assumed to represent the same core trimmed to slightly different extents by micrococcal nuclease. Band intensities indicate the fraction of cores at each position, but a more accurate estimate was obtained by measuring the fraction of core DNA remaining after digestion with each enzyme. Marker (M), end-labeled MspI digest of pBR322. Arrowheads indicate the bands attributed to the major core before or after transcription. The bands deriving from each nucleosome core before transcription are as follows. Major core (complex 2): Lane B, 44 and 104 bp; lane E, 65 and 83 bp bands. Minor core (complex 3): lane B, 81 and 69 bp; lane E, 120 and 30 bp. Another core: lane A, 32, 36 bp and 113, 117 bp; lane B, 115, 119 bp and 30, 35 bp. After transcription, the bands deriving from each nucleosome core are as follows. The major core: lane A, 20 and 130 bp; lane B, 103 and 47 bp. Another major core: lane A, 32, 36 bp and 113, 118 bp; lane B, 115, 119 bp and 30, 35 bp. Minor core: lane B, 81 and 69 bp; lane E, 120, 30 bp.

(B) Summary of nucleosome core positions before and after transcription. The cores drawn above the restriction map are those observed before transcription; the cores drawn below the map are those observed after transcription. The major positioned core before transcription (complex 2) and the two major cores after transcription are drawn in bold outline; minor positioned cores (including complex 3) are outlined with thinner lines. The positions are accurate to ±5 bp.

the result either of translocation of the nucleosome core to a symmetrically related position on the template (to account for the unaltered mobility) or of a conformational transition within the transcribed nucleosome core. To distinguish these possibilities, the experiment was repeated with the restriction enzymes AvaI and ScaI, both of which cleave near the promoter (Figure 1A). If nucleosome core translocation occurs, sensitivity to these enzymes before transcription and protection afterwards is expected (opposite to HaeII). A conformational change in the nucleosome core induced by transcription should not affect AvaI or ScaI digestion, because both restriction sites are outside the core. Before transcription, most of complex 2 was sensitive to ScaI or AvaI, yielding nucleosome cores of higher mobility, owing to removal of some DNA external to the core (Figure 1B, lanes 5, 6, and 7). After transcription, nearly all of the nucleosome cores were resistant to both enzymes (lanes 8, 9, and 10). In all cases free template was cleaved as expected. Thus, transcription resulted in translocation of the nucleosome core from the vicinity of the HaeII site to the vicinity of the AvaI and ScaI sites. These changes in sensitivity to restriction enzymes required transcription; they were not observed if one of the ribonucleotide triphosphates (rNTPs) or polymerase was omitted (data not shown). Similar results were obtained for the hexamer complex (Figure 1C).

To confirm that translocation is induced on transcription and to determine nucleosome core positions directly, nucleosomal templates were digested to core particles with micrococcal nuclease. Core DNA (~146 bp) was isolated, labeled at both ends, and digested with various restriction enzymes to map nucleosome core positions (Figure 2) as described (Clark and Felsenfeld, 1992). Before transcription, most of the cores were positioned with one boundary very close to the NcoI end of the template. The other cores occupied one of two minor positions, one at the promoter end of the template, symmetrically positioned with respect to the major core, and the other at the center of the template (complex 3) with the promoter at its border. After transcription, two major positioned cores differing by ~5 bp in translational position appeared at the SacI end of the template. Both of these existed as minor cores before transcription (one almost undetectable and therefore not shown in Figure 2B). In contrast, the major nucleosome core present before transcription became a minor core after transcription. The minor cores on

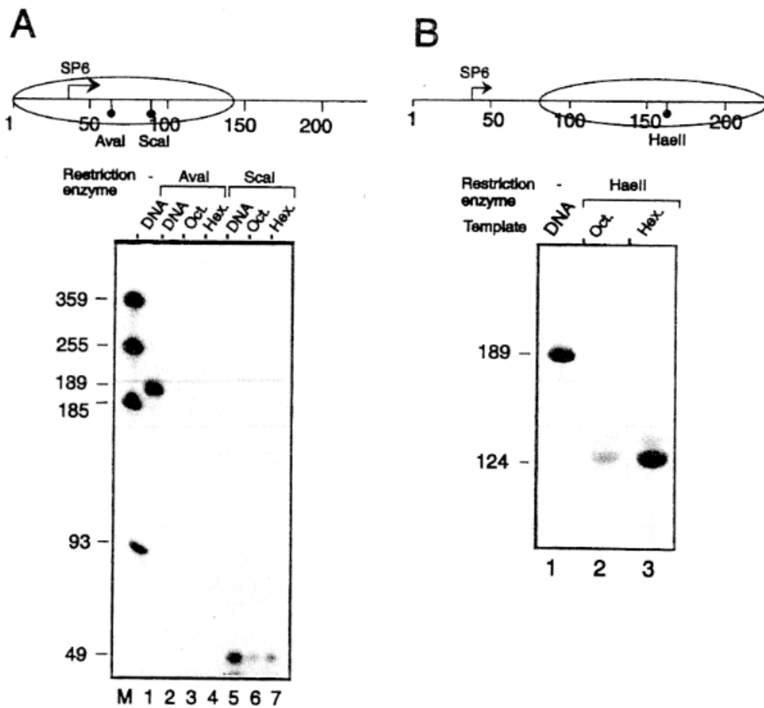

Figure 3. Nucleosome Core Translocation Occurs during the First Round of Transcription and Results in Promoter Blockage

(A) Translocation of the nucleosome core during transcription results in promoter blockage; analysis of labeled transcripts in a denaturing gel. Cores were transcribed for 10 min in the absence of labeled nucleotide to induce translocation to the promoter region such that the ScaI and AvaI sites were protected from digestion (as shown in the diagram). Sufficient AvaI or ScaI was added to digest all unprotected templates (30 min). [α-^{32}P]UTP and fresh polymerase were added to label all transcripts synthesized after restriction enzyme digestion, and transcription was continued for 20 min. Transcripts were derived from the following: free template without restriction digestion (lane 1), free template (lanes 2 and 5), cores (lanes 3 and 6), and hexamer complexes (lanes 4 and 7) after digestion with AvaI or ScaI, as indicated. Marker (M), runoff transcripts obtained by transcription of a mixture of restriction digests of pB19A.

(B) Nucleosomal templates are transcribed only once; analysis of transcripts in a denaturing gel. Cores were digested with 5 U of HaeII for 1 hr at 37°C in transcription buffer to cleave all templates with an exposed HaeII site. Only those templates with a core protecting the HaeII site (as shown in the diagram) will give rise to full-length transcripts. Polymerase was added; transcription was for 20 min. Lane 1, free template; lane 2, cores digested with HaeII; lane 3, hexamer complexes digested with HaeII. The bands were excised from the gel and the number of transcripts synthesized was determined by scintillation counting.

and next to the promoter appeared to be stable to transcription, presumably because the promoter is blocked (Lorch et al., 1987; see below).

Digestion of hexamer complexes with micrococcal nuclease gave protected fragments of 105–110 bp. Restriction analysis of this DNA revealed a single hexamer position right at the NcoI end of the template (data not shown). Presumably this complex contains a hexamer rather than an octamer, because, in the absence of dimer–DNA contacts, the interaction of the dimer with the hexamer is too weak. After transcription, the hexamer complex gave a DNAase I footprint consistent with its translocation to the promoter end of the template (data not shown).

In summary, micrococcal nuclease mapping and restriction enzyme protection (and DNAase I footprinting, data not shown) demonstrate that transcription induced a translocation of the nucleosome core from one end of the template to the other, a distance of 75 or 80 bp.

Translocation of the Nucleosome Core Blocks Transcription

A nucleosome core formed on an SP6 promoter completely blocks transcription (Lorch et al., 1987). Therefore, translocation of the core to a position over the promoter should prevent further transcription. This was tested (Figure 3A). Nucleosome cores were transcribed to induce translocation, and then AvaI or ScaI was added to digest all templates not having a core protecting these sites (the extent of digestion was checked in a nucleoprotein gel; data not shown). Only templates with a nucleosome core over the promoter survive intact (see Figure 2B), and if these can be transcribed they should yield full-length transcripts (189 nt). Labeled UTP was then added, transcription continued, and the labeled transcripts analyzed. Templates digested with ScaI gave short transcripts only (template digested with AvaI yielded a very short transcript that ran off the bottom of the gel). No full-length transcripts were observed after digestion with either enzyme. Thus, translocation of the nucleosome core to new positions over the promoter was sufficient to block transcription.

Does translocation occur during the first round of transcription? If so, a nucleosomal template should yield only one transcript before being repressed. Nucleosome cores were digested with just enough HaeII to cleave all templates without a core over the HaeII site (i.e., free templates and templates with a core over the promoter) in 1 hr (Figure 3B). Polymerase was added, and transcription was allowed to proceed for 20 min. Full-length transcripts should be synthesized only from templates that originally had a core protecting the HaeII site; templates cleaved by HaeII should give shorter transcripts (124 nt). About 0.8 full-length transcripts per template were obtained, corresponding to ~1.1 transcripts per nucleosomal template (after correction for the 30% of cores already positioned over the promoter and therefore never transcribed). In contrast, many transcripts (124 nt) were synthesized from templates that did not have a protected HaeII site. Thus, translocation occurred during the first round of transcription.

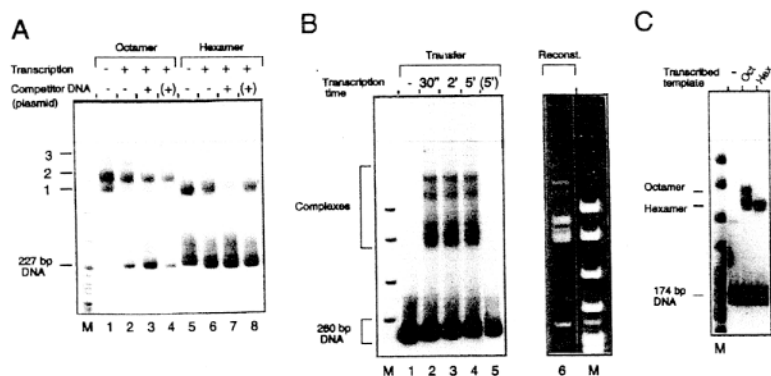

Figure 4. The Histone Octamer Can Be Transferred to Competitor DNA (Transfer in Trans)

(A) Loss of histone octamers from nucleosome cores during transcription in the presence of competitor DNA; analysis in a nucleoprotein gel. Cores were mixed with plasmid pBS1100 at 50 μg/ml (170-fold excess in moles of base pairs) and transcribed for 20 min. Lane 1, no competitor and no polymerase; lane 2, polymerase added, no competitor; lane 3, polymerase and competitor present; lane 4, competitor added 30 s after polymerase and transcription continued for another 20 min. Lanes 5–8, the same experiment using hexamer complexes. Marker (M), end-labeled MspI digest of pBR322.

(B) Octamer transfer to competitor DNA during transcription results in the formation of positioned nucleosome cores. Analysis of complexes formed on a competitor DNA fragment in a nucleoprotein gel. Unlabeled cores were mixed with an end-labeled 260 bp competitor fragment (containing a strong nucleosome-positioning sequence) and transcribed for up to 5 min as indicated. Lane 1, before transcription; lanes 2–4, after transcription for 30 s, 2 min, and 5 min, respectively. Lane 5, competitor added 30 s after polymerase and transcription continued for another 5 min. Lane 6, analysis of cores reconstituted on the 260 bp fragment by salt and urea dialysis (the gel was stained afterwards with ethidium bromide). Marker (M), MspI digest of pBR322.

(C) The histone hexamer is transferred to a competitor DNA fragment without dissociation to tetramer and dimer. Analysis of complexes formed on competitor fragments in a nucleoprotein gel. Unlabeled cores or hexamer complexes were mixed with a 174 bp end-labeled DNA fragment derived from the 227 bp template by removal of the promoter region. Marker (M), end-labeled MspI digest of pBR322.

A Choice for the Histone Octamer: Transfer in Cis or in Trans

Is the nucleosome core translocated within the same complex (transfer in cis) or is it transferred to another DNA fragment (in trans)? To investigate this, the ability of transcriptionally inert competitor DNA to capture histones during transcription was examined.

Labeled nucleosome cores were mixed with a large excess of negatively supercoiled plasmid as competitor, transcribed, and analyzed in a nucleoprotein gel (Figure 4A). In the absence of transcription, competitor DNA had no effect on the stability of the core (data not shown). In the presence of competitor, much more free template was liberated during transcription than in the absence of competitor (compare lanes 2 and 3). When competitor DNA was added 30 s after transcription had been initiated, excess free template was not liberated, and the nucleosome core was stable (lane 4), indicating that translocation to promoter-blocking positions was complete before the addition of competitor DNA. Therefore, the octamer was available for transfer in trans for less than 30 s, the time required to synthesize about one transcript per template. The fraction of octamers transferred to competitor as a function of competitor DNA concentration increased over the range 0.3 to 50 μg/ml, reaching a plateau of 50% transfer in the range of 20 to 50 μg/ml. Of the cores remaining on the template, 30% of the total were already positioned over the promoter (untranscribable), and the other 20% were translocated to positions over the promoter (translocation was demonstrated using the restriction enzyme protection assay; data not shown). Therefore, ~30% of cores on transcribable nucleosomal templates were translocated in cis even in the presence of a very large excess of competitor.

Transcription of free template was inhibited by ~10% in the presence of competitor DNA at 50 μg/ml, probably owing to nonspecific binding of polymerase. In contrast, addition of competitor DNA resulted in the synthesis of many more transcripts from both nucleosome cores and hexamer complexes, reflecting multiple rounds of transcription from templates liberated by transfer of the octamer or hexamer to competitor (data not shown).

Is the octamer transferred to competitor DNA? Unlabeled nucleosome cores were mixed with a labeled 260 bp DNA fragment containing a single Xenopus somatic 5S RNA gene as competitor (Figure 4B). This fragment was chosen because the various positioned cores formed during reconstitution had been characterized previously (Clark and Felsenfeld, 1992). After transcription, the complexes formed on the 260 bp fragment were analyzed in a nucleoprotein gel and compared with those obtained after reconstitution on the same fragment by salt and urea dialysis. The complexes formed with labeled competitor DNA were very similar to those observed after reconstitution. Thus, octamers transferred to competitor DNA formed nucleosome cores at different positions. The time course of core formation on the competitor fragment showed that octamer transfer was complete within 30 s. Under these conditions, using an equimolar quantity of competitor, transfer in cis is approximately 4-fold more likely than transfer in trans (data not shown).

Does transfer to competitor involve free histone tetramer and dimers? The octamer is very unstable at low salt concentrations in the absence of DNA, dissociating to an H3/H4 tetramer and two H2A/H2B dimers (Thomas and Kornberg, 1975; Eickbush and Moudrianakis, 1978). If octamer transfer begins with its displacement from the template into solution, dissociation into a tetramer and two dimers might occur. Then re-formation of a nucleosome core would require two dimers separately to locate and

Figure 5. The Histone Octamer Is Transferred Only in Cis at Low Transcription Rate

(A) The fraction of octamers transferred to competitor DNA depends on the concentration of rNTPs. Nucleosome cores were mixed with competitor DNA (chicken erythrocyte core particle DNA at 50 μg/ml) in the presence of different concentrations of rNTPs, transcribed, and analyzed in a nucleoprotein gel. The fraction of cores that dissociated to histone-free template was plotted as a function of [rNTP] (closed squares). Also plotted is the rate of transcription (transcripts synthesized per template in 20 minutes) of free template as a function of [rNTP] (open squares).

(B) The octamer cannot be transferred to competitor at low transcription rate. Analysis in a nucleoprotein gel. Cores were mixed with competitor DNA (3, 10, 30, 100, and 300 μg core DNA/ml) and transcribed for 1 hr (to ensure that at least one transcript was synthesized per template at 0.1 mM rNTP) in the presence of either 0.5 mM or 0.1 mM rNTPs. Marker (M), end-labeled MspI digest of pBR322.

(C) Transfer of the histone octamer occurs at both high and low rates of transcription. Analysis in a nucleoprotein gel using the restriction enzyme protection assay. Nucleosome cores were mixed with competitor DNA (chicken erythrocyte core particle DNA at a final concentration of 50 μg/ml) and rNTPs at either 0.5 mM or 0.1 mM, as indicated, and incubated in the presence or absence of SP6 RNA polymerase for 1 hr. Templates were then digested with HaeII as indicated. Note: the 70 bp fragment generated by HaeII digestion of the template ran off the gel. Marker (M), end-labeled MspI digest of pBR322.

bind to a tetramer–DNA complex. This model was tested using the hexamer complex; if the hexamer is displaced and decomposes to tetramer and dimer, octamers and tetramers as well as hexamers would be detected on competitor DNA, but if the hexamer is transferred directly, without dissociation, only hexamers should be detected. Unlabeled nucleosome cores or hexamer complexes were mixed with a labeled 174 bp XhoI–NcoI fragment as competitor, transcribed, and analyzed in a nucleoprotein gel (Figure 4C). The 174 bp fragment was derived from the 227 bp template by removal of a promoter-containing fragment and has the same octamer and hexamer positioning signals (data not shown). In the absence of transcription, no complexes were formed on the competitor fragment. Transcription of cores resulted in the formation of cores and hexamer complexes on the competitor fragment. This result can be explained if the transferred octamer selected either the octamer or the hexamer position, but the choice of the latter resulted in the subsequent loss of a dimer. Transcription of hexamer complexes resulted only in hexamer complexes. Therefore, the hexamer does not dissociate to tetramer and dimer during transfer. This is consistent with the observation that transcription through a nucleosome core by T7 RNA polymerase does not require dissociation of the octamer to a tetramer and two dimers (O'Neill et al., 1993).

In summary, transcription can induce transfer of the octamer in trans to re-form positioned nucleosome cores. However, transfer in cis is predominant. This could in principle reflect either indirect transfer (the octamer is displaced into solution and recaptured on the same template molecule, owing to its high local concentration) or direct transfer (the octamer is transferred without leaving the template). This question is resolved below.

The Octamer Is Transferred Only in Cis at Low Transcription Rate

The effect of decreasing the rate of transcription on the fraction of octamers transferred in trans was investigated. The rate of transcription of nucleosome-free template fell by a factor of 30 when the rNTP concentration was reduced from 0.5 mM (the concentration used in all the experiments described above) to 0.1 mM (Figure 5A). This decrease in the number of full-length transcripts synthesized might reflect a large increase in the fraction of transcripts aborted soon after initiation, when they are less than 7 nt long (Nam and Kang, 1988). We were not able to measure average transcript elongation rates at 37°C, because they were too high, but at 20°C the rate at 0.1 mM rNTPs was about half that at 0.5 mM rNTPs (unpublished data). Thus, the polymerase takes about twice as long to synthesize a full-length transcript at 0.1 mM rNTPs than at 0.5 mM rNTPs.

Labeled nucleosome cores were transcribed at different rNTP concentrations in the presence of competitor DNA and analyzed in a nucleoprotein gel. The fraction of octamers transferred to competitor depended steeply on [rNTP] (Figure 5A) and correlated very closely with the dependence of the transcription rate on [rNTP], sug-

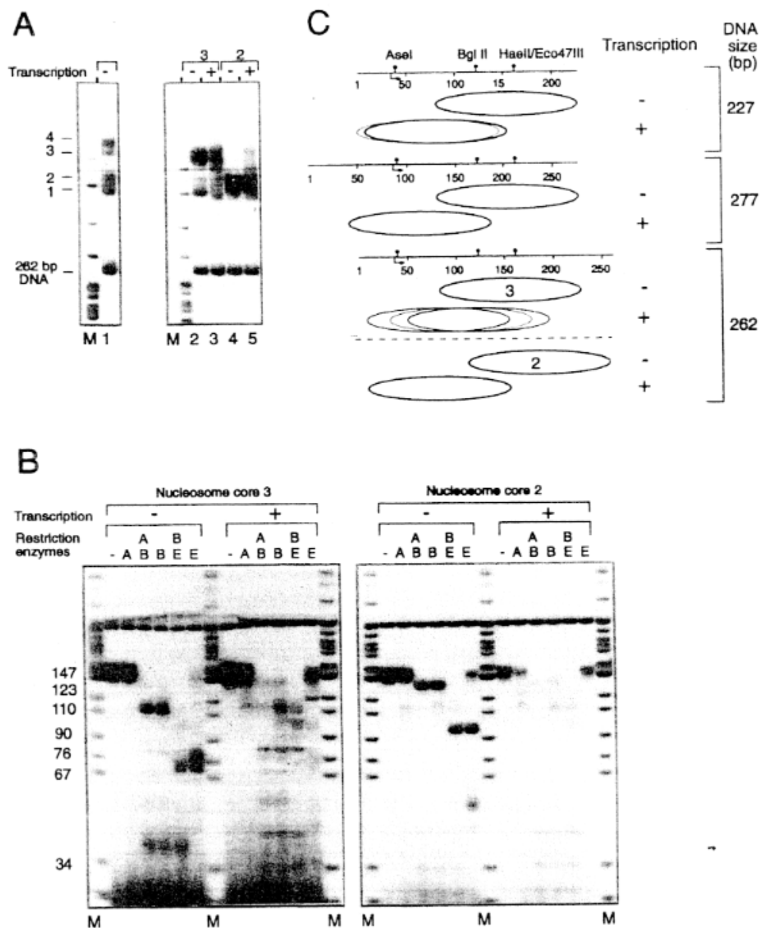

Figure 6. The Position of a Nucleosome Core after Transcription Depends on Its Position before Transcription

(A) Transcription of two different positioned nucleosome cores on the 262 bp template; analysis in a nucleoprotein gel. Lane 1, resolution of four complexes formed on the 262 bp template after reconstitution in a preparative nucleoprotein gel. Uniquely positioned nucleosome cores 2 and 3 (as indicated) were isolated from a preparative gel and incubated with (lanes 3 and 5) or without (lanes 2 and 4) polymerase. Marker (M), MspI digest of pBR322.

(B) The fates of two isolated nucleosome cores on transcription; micrococcal nuclease mapping. After incubation for 2 hr with or without polymerase at 0.1 mM rNTPs, the templates were digested to core particles with micrococcal nuclease. Core positions were mapped as described in the legend to Figure 2. The fates of both cores are shown in (C). Note that about 20% of core 2 and about 10% of core 3 (Table 1) remained at their original positions after transcription, presumably because these templates were not transcribed. The gels were quantitated using a phosphorimager; recoveries relative to undigested core DNA were determined using the 245 bp control fragment to correct for losses and were generally high (90% or more). Very short fragments that migrated off the gel or migrated in the smear at the bottom of the gel could not be quantitated; in lanes where cores giving rise to such fragments accounted for a significant fraction of the total, the measured recoveries were correspondingly lower.

(C) Summary of the fates of several transcribed nucleosome cores. The three templates described in the text (227 bp, 277 bp, and 262 bp) are aligned to reflect their common DNA sequence content. Relative to the 227 bp template, the 277 bp template is extended by 50 bp at the promoter end, and the 262 bp template is extended by 35 bp at the other end. The SP6 promoter is indicated with an arrow. The diagram shows only the quantitatively major cores that are translocated on transcription; for each template, the upper core indicates the position before transcription, and the lower core(s) indicates the position(s) after transcription. Nucleosome cores 2 and 3 formed on the 262 bp template are indicated.

gesting that the availability of an octamer for transfer in trans depends on the transcription rate. As shown above, up to ~50% of all octamers were transferred to competitor DNA on transcription with 0.5 mM rNTPs (lanes 3–7). In contrast, transfer in trans did not occur with 0.1 mM rNTPs, even at extremely high concentrations of competitor DNA (Figure 5B; lanes 8–12). Nucleosome core translocation occurred at both rNTP concentrations, as judged using the restriction enzyme protection assay (Figure 5C). Transfer was complete at both rNTP concentrations after the synthesis of one transcript per template (data not shown).

Thus, the octamer could not be transferred to competitor DNA at low rNTP concentration; octamers were transferred only in cis under these conditions, regardless of the amount of competitor DNA present. The indirect transfer (release/recapture) model attempts to account for these results by requiring that the octamer be released into solution and then recaptured by the original template DNA, because of the high local charge concentration of the template. It is hard to imagine in any case that large excesses of competitor would fail to capture some octamers under such circumstances. However, this model is ruled out altogether by the results shown in Figure 5, because the ability of the octamer to transfer to competitor depends on rNTP concentration, even though the effective local concentration of template could not have changed as the rNTP concentration was raised. We conclude that the transfer of the octamer in cis at low rNTP concentration occurs by a direct mechanism; that is, the octamer does not leave the template. As we will show in the Discussion, transfer in trans observed at higher rNTP concentrations is also consistent with a direct transfer through collision without release of the octamer into solution. Below, we provide independent evidence that transfer is direct.

The Final Position of a Nucleosome Core Depends on Its Initial Position

Does the position of a nucleosome core after transcription depend on its position before transcription? To answer this question, it was necessary to isolate two different cores positioned on the same template, transcribe them, and compare their final positions. For this experiment, a derivative of the 227 bp template with a 35 bp extension at the NcoI end (to the HindIII site) was used. This 262 bp tem-

plate was expected to form two different nucleosome cores, one at the same position as the major core on the 227 bp template (see Figure 2B) and the other at a position at the HindIII end of the template corresponding to that of the hexamer on the 227 bp template.

Nucleosome cores were reconstituted on the labeled 262 bp template and applied directly to a preparative nucleoprotein gel (Figure 6A, lane 1). Four different complexes were resolved, isolated from the gel, and characterized by digestion with micrococcal nuclease. Complexes 2 and 3 yielded protected DNA fragments of ~146 bp (see below) and therefore contained nucleosome cores. Complex 1 resulted in protected fragments of ~110 bp and is probably a hexamer complex. Complex 4 was refractory to digestion, suggesting that it is completely histone-covered.

Examination of isolated complexes 2 and 3 in a nucleoprotein gel (Figure 6A) revealed that both contained significant amounts of free DNA, presumably from complexes destroyed during the isolation procedure. However, the remaining complexes were intact because they had the same mobilities after isolation as they did before (compare lanes 2 and 4 with lane 1). Transcription of nucleosome core 3 resulted in several different complexes, indicating that translocation to several different positions had occurred. In contrast, transcription of core 2 gave a band of the same mobility, suggesting translocation to a single position symmetrically related to its position before transcription. Restriction enzyme protection analysis confirmed that translocation had occurred (data not shown).

The positions of the nucleosome cores before and after transcription were determined using micrococcal nuclease (Figures 6B and 6C), and the fraction of cores at each position was quantitated using a phosphorimager (Table 1). Before transcription, core 3 occupied the same DNA sequence as the major core formed on the 227 bp template. Core 2 was positioned right at the HindIII end of the 262 bp template; as expected, it occupied the same DNA sequence as the hexamer complex formed on the 227 bp template, and the extra 35 bp in the 262 bp template were used to complete the core. After transcription, core 3 gave rise to a set of three positioned cores at the promoter end of the template, corresponding to translocations of 75 bp, 60 bp, and 40 bp, consistent with the mobilities of the complexes observed in the nucleoprotein gel (Figure 6A). In contrast, core 2 gave rise to just a single core at an almost symmetrical position at the promoter end of the template, a translocation of 95 bp. Clearly, the final position of the nucleosome core depends on its initial position, but apparently not in a simple way (see below).

Does the final position of the nucleosome core depend on the length of DNA available for translocation? A derivative of the 227 bp template was prepared with an extra 50 bp at the promoter end (total length, 277 bp), and reconstitutes were analyzed in the same way as the 227 bp template (data not shown). Micrococcal nuclease mapping revealed two cores at symmetrically related positions at the ends of the template, and two quantitatively minor positioned cores. As expected, the major core at the promoter end of the template was unaffected by transcription, because the promoter was blocked. Transcription induced translocation of the core at the NcoI end of the template over 90 bp to a new position with its 5′ border ~45 bp from the BstNI end of the template (Figure 6C). This new position coincided with a very minor position before transcription. The highly favorable position at the promoter end of the template was ignored in favor of a previously minor position, suggesting that the mechanism of translocation limits the choice of DNA-binding sites for the octamer. The translocated core utilized only 5 bp of the 50 bp extension of the template.

The major nucleosome core on the 227 bp template, core 3 on the 262 bp template, and the transcribed major core on the 277 bp template all contained the same DNA sequence, but their fates on transcription were different (Figure 6C). The three cores differ only in the lengths of DNA extending on either side of the core. The core on the 227 bp template was situated right at the end, and transcription induced a translocation of 75 or 80 bp to within a few base pairs of the other end of the template. The core on the 277 bp template was translocated slightly farther (90 bp) to a different position; perhaps the extra DNA modifies the sequence signal directing the octamer to its final position. The core on the 262 bp template was translocated by 75, 60, or 40 bp to one of three different positions, implying that the extra 35 bp of DNA on the side of the core distal from the promoter somehow influences the destination of the octamer (see below). This result also suggests that the location of the transcribing polymerase on the template molecule at the moment of octamer displacement is not important in deciding the fate of the octamer, because in all three cases the distance between the promoter and the core is the same.

The experiments with the 262 and 277 bp templates show that the position of a nucleosome core after translocation depends on its position before transcription, that translocation occurs over a distance of 40–95 bp, and that

Table 1. Quantitative Analysis of Nucleosome Cores Positioned on Templates 2 and 3 before and after Transcription

Template	Nucleosome Core[a]	Fraction of Total Cores (%)[b] – Transcription	+ Transcription
2	2	81	19
	2A	7	72
	Minor	12	9
3	3	86	8
	3A	0	48
	3B	0	12
	3C	6	22
	Minor	8	10

[a] The positions of the nucleosome cores are shown in Figure 6C. Nucleosome core 2A is the major core on template 2 after transcription. Nucleosome cores 3A, 3B, and 3C are the major cores on template 3 after transcription (from left to right).

[b] All lanes in Figure 6B were scanned using a phosphorimager; the fractions of total cores were calculated from lanes for which recovery of counts was >90% with respect to undigested core DNA. We estimate that the error in these numbers is of the order 10%. Numbers for minor positions were obtained by subtracting the sum of the numbers for major positions from 100%.

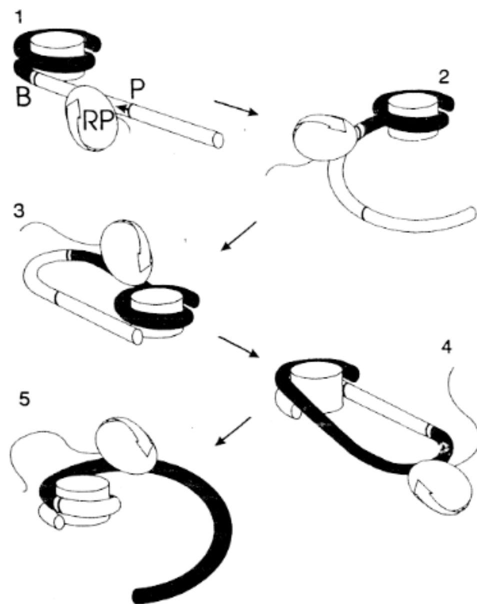

Figure 7. A Spooling Mechanism for Transcription through a Nucleosome Core

(1) RNA polymerase (RP) initiates transcription at the promoter (P) on the 227 bp template and begins to synthesize a transcript; the border (B) of the nucleosome core is indicated by a bar, and nucleosomal DNA is shaded. The drawing is roughly to scale. (2) As the polymerase approaches the core it induces the dissociation of proximal DNA from the core, exposing part of the octamer surface. (3) The DNA behind the polymerase binds to the exposed octamer surface to form a loop within the nucleosome core to which the transcribing polymerase is still bound; this loop is a topologically isolated domain subject to superhelical stress. (4) The DNA ahead of the transcribing polymerase continues to uncoil from the octamer and the DNA behind the polymerase begins to coil around the octamer. (The polymerase is drawn bound on the outside of the bend, but note that the direction of polymerase-induced DNA bending is not known.) (5) The nucleosome core is reformed behind the polymerase (blocking the promoter), and the polymerase completes the transcript.

the destination of the octamer is influenced by local positioning signals in DNA. These observations rule out transient dissociation of the octamer from DNA during translocation, since information about previous location would be lost, and different fates for different nucleosome cores would not be possible. When the 262 bp nucleosomal templates were transcribed in the presence of 0.5 mM rNTPs in the absence of competitor, the fates of the octamers were the same (data not shown), indicating that the mechanism of transfer in cis is the same at high and low transcription rates.

Discussion

A Model for Transcription through a Nucleosome Core

Our results show that transcription induces translocation of a nucleosome core over a distance of 40–95 bp by a direct transfer mechanism. How does translocation occur if neither the octamer nor the polymerase leaves the template during transcription? Clearly, the octamer must somehow step around the transcribing polymerase, always maintaining contact with DNA. Transcription must induce the formation of a transient bridging complex in which the octamer is in contact with both donor DNA (DNA at its original position) and acceptor DNA (DNA at the new position), with the transcribing polymerase in between the two sets of histone–DNA contacts. A bridging complex would be energetically favored relative to a free octamer intermediate, because histone–DNA contacts are broken sequentially rather than simultaneously.

We propose a model based on the structure of the nucleosome core (Richmond et al., 1984) that accounts for our observations: a spooling mechanism for octamer translocation (Figure 7). First, we will discuss transfer in cis. As the transcribing polymerase approaches the nucleosome core, it causes proximal DNA to begin uncoiling from the surface of the histone octamer. As the polymerase continues to invade the nucleosome core, more DNA uncoils from the octamer, and the DNA behind the polymerase is captured on the exposed octamer surface (this interaction might be facilitated by polymerase-induced DNA bending in the elongation complex [Heumann et al., 1988]), resulting in a DNA loop within the nucleosome core. In this bridging complex, the transcribing polymerase is bound to the internal loop. The DNA ahead of the transcribing polymerase continues to uncoil from the octamer as the DNA behind it begins to coil around the octamer. Thus, the nucleosome core is translocated to its new position and the polymerase is able to complete the transcript.

The formation of an internal loop would create ideal conditions for transcription-induced supercoiling (Liu and Wang, 1987); both ends of the DNA loop are tethered (and so unable to rotate to release stress), and the polymerase is probably sterically inhibited from rotating around the DNA. Positive supercoiling ahead of the polymerase would drive the uncoiling of nucleosomal DNA, and negative supercoiling behind the polymerase would drive the recoiling of DNA onto the octamer. Sufficient positive superhelical stress to uncoil the DNA remaining on the nucleosome core would be generated after transcription of only a few base pairs once the loop is formed, requiring prompt resolution of the bridging intermediate, which might therefore be very short-lived. Thus, the energetically highly favorable transfer of an octamer from positively supercoiled to negatively supercoiled DNA (Clark and Felsenfeld, 1991) might provide the activation energy for translocation.

Mechanism of Transfer in Trans

Does transfer in trans occur directly (by collision with a competitor DNA molecule) or indirectly (via octamer release/recapture)? In the absence of competitor, most octamers are transferred directly in cis even at high rNTP concentration, but in the presence of increasing amounts of competitor DNA, increasing numbers of these octamers are transferred in trans. Therefore, octamers that were committed to direct transfer on the same template molecule (in cis) can be diverted to competitor DNA (in trans), to an extent determined by the competitor concentration.

In other words, an octamer is vulnerable to competitor DNA during direct transfer. This implies that competitor DNA must interact directly with the transfer intermediate for transfer to occur in trans and that transfer in trans probably also proceeds via a direct mechanism. The observed plateau at 70% in the fraction of octamers transferred at high competitor concentration (see above) is entirely consistent with this model and inconsistent with the release/recapture model. If the octamers were free in solution, raising the competitor:template ratio should continue to raise the fraction of octamers transferred. On the other hand, if transfer occurs through formation of a ternary complex involving template DNA, competitor DNA, and octamer, it is easy to see how the outcome could be independent of the competitor concentration once the system was saturated for ternary complex formation.

Transfer in trans is possible only at high rNTP concentration. How does the rNTP concentration influence the fate of the octamer? The reduction in transcription rate at 0.1 mM rNTPs is probably largely due to changes in the fraction of transcripts aborted just after initiation, but it is difficult to envisage how this could prevent octamer transfer in trans. For this reason, we suggest that it is the more modest change in the transcript elongation rate (i.e., the rate at which the polymerase proceeds down the template) that is important in determining the fate of the octamer. Transfer in trans may now be accounted for in terms of the model depicted in Figure 7: at high rNTP concentration, the polymerase transcribes faster and displaces DNA from the octamer more rapidly, exposing more of the octamer surface. For transfer in trans, competitor DNA must bind to this surface before the DNA behind the polymerase binds. Alternatively, the DNA ahead of the polymerase might uncoil from the octamer faster than the DNA behind it coils around the octamer, resulting in an exposed octamer surface on the distal side of the nucleosome core. Competitor DNA might bind to this surface and begin to coil around the octamer, competing with the re-coiling of template DNA.

What happens to a nucleosome core on a longer template? Previously we showed that a core formed at a defined site on a plasmid was transferred to other sites on the plasmid during transcription (transfer in cis), with a preference for the ~900 bp region behind the promoter (Clark and Felsenfeld, 1992). These results can be explained using the model in Figure 7 by postulating a variable loop size: the destination of the octamer is determined by the DNA segment behind the polymerase that contacts the exposed octamer surface. A very short template has few acceptor sites, but a longer template (linear or circular) has many. It seems likely that the topology of the plasmid template at the moment of transfer will determine the destination of the octamer (Clark and Felsenfeld, 1992).

The Distance of Translocation: The Length of the Step

The distances of translocation observed varied from 40 bp to 95 bp and averaged 74 bp. However, nucleosome core 3 on the 262 bp template exhibited both of the shortest distances of translocation observed (40 and 60 bp). In this case, the octamer chose from one of three destinations, corresponding to translocations of 75, 60, and 40 bp. A possible explanation for this observation (and for the conclusion that DNA extending from the side of the core away from the promoter influences the destination of the octamer) is that the approaching polymerase sometimes causes the octamer to slip downstream toward the end of the template before it is translocated in the opposite direction (this would not happen to the core on the 227 bp template because it is already at the end). In terms of the spooling mechanism (Figure 7), slippage represents a rotation of the octamer with respect to the untranscribed DNA in the bridging complex. If this explanation is correct, the 60 and 40 bp translocations reflect octamer slippage of ~20 and ~30 bp, respectively, prior to translocation in the opposite direction; the 75 bp translocation occurs without slippage, and the actual distances of translocation would be ~70–80 bp in all three cases. We have no evidence for octamer slippage, but we are investigating this problem. If the translocations of 40 and 60 bp are disregarded, the average of all other observed translocations is 83 bp (which coincides with the number of base pairs in a complete turn of nucleosomal DNA).

Other Studies of Transcription through a Nucleosome Core on a Short Template

The question of the fate of a nucleosome core on a short template after transcription by SP6 RNA polymerase has been controversial; Lorch et al. (1987, 1988) concluded that the core was lost from the template after the first round of transcription, but Losa and Brown (1987) concluded that it stayed in place. It was suggested that a core formed on the 5S DNA positioning sequence used by Losa and Brown (1987) behaves differently from bulk cores (Lorch et al., 1988). However, in our earlier study (Clark and Felsenfeld, 1992), a core formed on the same 5S DNA was displaced on transcription. Furthermore, a nucleosome core formed on the 5S DNA template used by Losa and Brown (1987) was also translocated on transcription (unpublished data). Thus, in no case do we find that the nucleosome core remains in place after transcription. As we have shown, transfer always occurs either to another site on the same template or to added competitor DNA, depending upon the conditions.

Transcription of Eukaryotic Genes

SP6 RNA polymerase was used in this study because it is available highly purified and it is extremely efficient (i.e., all the templates can be transcribed). In contrast, eukaryotic RNA polymerases are very inefficient in vitro, making a quantitative analysis extremely difficult. SP6 RNA polymerase is a phage enzyme that is obviously not designed to transcribe nucleosomal DNA, but it is able to do so with apparent ease. For this reason, we suggest that the mechanism used by different RNA polymerases to transcribe through nucleosomes is likely to be essentially the same, reflecting an intrinsic property of nucleosome cores.

Eukaryotic RNA polymerase II (Young, 1991) is about five times larger than SP6 RNA polymerase (99 kd; Kotani et al., 1987), suggesting that the average dimensions of

polymerase II are 1.7 times longer than those of SP6 polymerase. The number of base pairs occupied by SP6 RNA polymerase in its elongation complex is unknown, but a stalled T7 RNA polymerase elongation complex has a DNAase I footprint of 15–21 bp (Sastry and Hearst, 1991) and is likely to be very similar. The polymerase II elongation complex has a DNAase I footprint of 40–55 bp (Linn and Luse, 1991) and probably contains bent DNA (Darst et al., 1991; Kornberg and Lorch, 1991). The internal loop postulated in our model is long enough to accommodate a polymerase II elongation complex, and steric restrictions on rotation would be expected to become more acute.

What might the consequences of our model be if it were to operate in vivo? Eukaryotic genes are assembled into linear arrays of nucleosomes. The formation of an initiation complex by RNA polymerase at the promoter would require the displacement of the nucleosome situated at or near the transcription initiation site at some point in the process. The passage of the transcribing polymerase along the gene would induce translocation of the second nucleosome to DNA vacated by the first nucleosome, and translocation of the third nucleosome to DNA vacated by the second, and so on (note that the nucleosomal phase will not be preserved unless the distance of translocation is equal to the repeat length). Multiple rounds of transcription would tend to deplete nucleosomes from the 3′ end of the gene, although nucleosomes could be transferred back to this region during transcription, or there could be concomitant nucleosome assembly. It is expected from this model that the nucleosomal organization of a gene during transcription should be dynamic, and this appears to be the case (e.g., Wu et al., 1979; Lohr, 1983; Lee and Garrard, 1991).

Experimental Procedures

DNA Fragments and Plasmids

The 227 bp SacI–NcoI fragment contains a modified SP6 polymerase promoter linked to a short fragment derived from the yeast *GLN3* gene and was prepared by digestion of pB22, constructed as follows: a 202 bp fragment was obtained by polymerase chain reaction amplification of a 168 bp region from the *GLN3* gene (nucleotides 2157–2324; Minehart and Magasanik, 1991) using two oligonucleotide primers (both 37 nt), CGCAGC**GGATCCTCGAG**TAGAAGGTCTTCAAGACGAA and CGCAGC**GGATCCCATGG**GCACTTACATTATTTGTTGT.

Both primers have 20 bases complementary to yeast DNA and tails of 17 nt containing restriction sites (in boldface: BamHI and XhoI or NcoI, respectively) to facilitate subsequent manipulation of the fragment. The 202 bp fragment was gel-purified, cleaved with BamHI, and ligated into the BamHI site of pUC19 to obtain pB17B (the XhoI end of the insert is nearer to the SacI site in pUC19 than the NcoI end). Two oligonucleotides, CTATCATACACATACGATTTAGGTGACACTATAGAATTAAT and TCGAGATAGTATGTGTATGCTAAATCCACTGTGATATCTTAATTA, were synthesized and annealed to obtain a minimal promoter for SP6 polymerase (designed using a consensus sequence; Brown et al., 1986) with a SacI end and a blunt end, and a site for AseI just downstream from the transcription start site (the last G in the upper [coding] strand).

The promoter fragment was attached to pB17B linearized with SmaI by blunt end ligation. The ligation products were digested with SacI to yield linear pB22 (with SacI ends), which was then circularized using ligase. The various short DNA fragments were obtained by digestion of pB22 with SacI and NcoI (227 bp) or SacI and HindIII (262 bp) or BstNI and NcoI (277 bp) or XhoI and NcoI (174 bp). The length of the 227 bp template was confirmed in a sequencing gel, but its mobility in a native gel was anomalous (it behaved as if it were ~240 bp; Figure 1B), suggesting that it contains a sequence-directed bend. The 260 bp XbaI–XmaI fragment containing a single copy of a Xenopus somatic 5S RNA gene was obtained from pXP10 (Wolffe et al., 1986). All fragments were purified by electroelution from agarose gels and end-labeled using Klenow fragment. The plasmid used as competitor was pBS1100 (3.8 kb; unpublished data). Core DNA was extracted from chicken erythrocyte core particles prepared as described (Ausio et al., 1989).

Reconstitution of Nucleosome Cores

Nucleosome cores were formed on short DNA fragments using the salt and urea dialysis method and separated from free DNA in sucrose density gradients as described (Clark and Felsenfeld, 1992). Usually, a ratio of ~0.8 histone octamer per fragment was used. Sucrose was removed from pooled fractions containing reconstitute using Centricon-10 (Amicon) pre-washed with buffer containing 5 μg bovine serum albumin (BSA) to reduce losses to the membrane; the final buffer was 10 mM HEPES (pH 8.0), 1 mM Na–EDTA, 0.1 mM phenylmethylsulfonyl fluoride. Cores were stored at 4°C and analyzed in 4.5% (40:1) polyacrylamide gels containing 20 mM HEPES (pH 8.0), 1 mM Na–EDTA, and 5% v/v glycerol (the running buffer did not contain glycerol) (Wolffe, 1987). Electrophoresis was for 3–5 hr at 100 V at room temperature. For preparative isolation of cores, the gel was preelectrophoresed for 8 hr to remove ammonium persulphate and TEMED. The reconstitute was made 10% v/v in sucrose and loaded; electrophoresis was for 7 hr at 100 V to maximize band separation. Bands located by autoradiography of the wet gel were excised and crushed and the cores eluted in two vol of 10 mM HEPES (pH 8.0), 1 mM Na–EDTA at 4°C in a tube on a rotator overnight. Gel pieces were removed by a low-speed microfuge spin, and concentrations of isolated cores were determined using the specific activity.

Transcription and Analysis of Nucleosome Core Positions

Typically, 0.02 μg template DNA in 20 μl was transcribed at 37°C with 40 U of SP6 RNA polymerase (80 U/μl, Promega) in 20 μl of 45 mM HEPES (pH 8.0), 6 mM $MgCl_2$, 2 mM spermidine, 5 mM 2-mercaptoethanol, 0.5 mM Na–EDTA, 0.1 mg BSA/ml, 0.05 mM phenylmethylsulfonyl fluoride, 1 U of RNAase inhibitor/μl (Boehringer), 0.5 mM (unless otherwise stated) each of ATP, UTP, GTP, and CTP, and 10 μCi [α-^{32}P]UTP at 3000 Ci/mmol (New England Nuclear). Omission of spermidine and BSA reduced the transcription rate by a factor of ~2 but did not affect the results. RNA was extracted, precipitated with ethanol, dissolved in formamide sample buffer, analyzed in an 8% (19:1) polyacrylamide gel, and compared with a set of marker SP6 polymerase runoff transcripts derived from a mixture of different restriction digests of pB19A (unpublished data). The number of transcripts synthesized was determined by excising bands from the gel and by scintillation counting. In experiments involving restriction digestion of templates before and after transcription, 10 U of enzyme was added, and incubation was for 30 min at 37°C; digestion was stopped by addition of Na–EDTA (pH 7.0) to a final concentration of 10 mM, and sucrose was added to 8% prior to analysis in nucleoprotein gels. To map core positions, unlabeled cores (0.1 μg DNA in 100 μl buffer) were transcribed and then digested with micrococcal nuclease as described (Clark and Felsenfeld, 1992). Core DNA was labeled using T4 polynucleotide kinase. Nucleoprotein gels and gels used to analyze restriction digestion of core DNA were quantitated using a phosphorimager.

Acknowledgments

We thank Tom Vandergon for donation of a fragment of the yeast *GLN3* gene, and Martin Gellert, Joan Boyes, and Emery Bresnick for valuable comments on the manuscript.

Received August 3, 1993; revised November 3, 1993.

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