The EMBO Journal vol.11 no.8 pp.2951 - 2959, 1992

Mobile nucleosomes—a general behavior

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Communicated by J.Palau

We have previously reported the mobility of positioned nucleosomes on sea urchin 5S rDNA. In this study we demonstrate the temperature dependence and the range of this mobility on 5S rDNA constructs. We find that this dynamic behavior also applies to bulk mononucleosomes and nucleosomes reconstituted onto sequences of the Alu family of ubiquitous repeats. We conclude that short range sliding is potentially a general phenomenon that is dependent on the underlying sequence and its position on the histone octamer. The nucleoprotein gel analysis used also reveals the dramatic effect on gel electrophoretic migration caused by the location of the histone octamer on DNA fragments. The usefulness of this technique for studying nucleosome positioning and its dynamics is demonstrated.

Key words: Alu family/chromatin/nucleosome mobility/ nucleosome positioning/5S rDNA/

Introduction

In the eukaryotic nucleus, the DNA is found packaged into nucleosomes, which are further organized into higher order chromatin structures. These orders of packaging of DNA are implicated in the accessibility of DNA sequence elements to *trans*-acting factors that control the processes of transcription and replication. They may, in addition, influence ongoing processes.

Recent lines of evidence support the idea that nucleosomes play a role in the regulation of transcription (reviewed by Grunstein, 1990 and Felsenfeld, 1992). In yeast, nucleosome shortage derepresses a number of genes *in vivo* (Han and Grunstein, 1988). For the specific cases of the yeast *PHO5* gene (Almer *et al.*, 1986; Fascher *et al.*, 1988) and the mouse mammary tumor virus long terminal repeat (Richard-Foy and Hager, 1987; Pina *et al.*, 1990) it has been reported that transcriptional activation is accompanied by removal of precisely positioned nucleosomes from the promoter to allow access of *trans*-acting factors to their cognate sequences.

Virtually all of the DNA in eukaryotic genomes is packaged in nucleosomes. It follows that nucleosomes have to be involved either passively or actively (or both) in the mechanisms of DNA processing. The roles of nucleosomes in these processes are far from clear but most probably involve dynamic properties such as nucleosome structural transitions, nucleosome unfolding and nucleosome mobility. Different modes of nucleosome disruption (Nacheva *et al.*, 1989; Lee and Garrard, 1991) as well as histone release (Jackson, 1990) during transcription have been suggested. Transfer of histone octamers from positively to negatively supercoiled DNA, away from the progressing transcription complex, has also been proposed (Clark and Felsenfeld, 1991). It is significant that DNA sequences can be designed that bind histone octamers more strongly than bulk nucleosomal DNA (Shrader and Crothers, 1989). It would appear that less than optimal binding strengths of histones for DNA sequences *in vivo* reflect the ability of histones to package the enormous variety of DNA sequences found in eukaryotic genomes. However, weaker binding could also confer the potential for nucleosome movement or removal.

Factors that should be expected to influence the dynamics of nucleosomes are the histone subtype composition and their states of reversible chemical modification. Histone hyperacetylation, among other histone modifications, has been linked to transcriptionally active states of chromatin (Hebbes *et al.*, 1988). It is thought to induce a more open nucleosome conformation, possibly facilitating the unfolding of higher order chromatin structures (reviewed by Csordas, 1990 and Bradbury, 1992). Similarly, histone H1, which is essential for the stabilization of higher order structures (Thoma *et al.*, 1979), is less abundant (or more easily lost) in active chromatin fractions (Ericsson *et al.*, 1990; Kamakaka and Thomas, 1990). H1 has been identified as a general repressor of transcription (Croston *et al.*, 1991).

In recent studies we have identified a dynamic behavior of positioned nucleosomes on sea urchin 5S rDNA. In low salt conditions, a temperature dependent redistribution of histone octamers was observed between a cluster of positions with the same rotational setting of the DNA (Pennings *et al.*, 1991; Meersseman *et al.*, 1991). This dynamic rearrangement of positioned nucleosomes should be distinguished from classical nucleosome sliding, which typically occurs over long distances, and exclusively at higher ionic strengths (Beard, 1978; Spadofora *et al.*, 1979).

The information available on DNA sequence and nucleosome positioning for sea urchin 5S rDNA allows detailed studies of the factors involved in short range nucleosome mobility in this system. Monomers and dimers of the 5S rDNA positioning sequence were used as substrates to determine the effects of temperature on nucleosome mobility and the limits or boundaries of these motions. Further, because the 5S rDNA sequence is exceptional in that it encodes a particularly dominant nucleosome binding site, we have investigated the short range nucleosome mobilities on the Alu family of sequence repeats and also the mobilities of native bulk mononucleosomes. We conclude that nucleosome mobility is potentially a general phenomenon, which has relevance to models of nucleosome involvement in DNA processing.

Results

We previously reported a direct link between the different positions of histone octamers on 207 bp of sea urchin 5S rDNA and the migrations of these positioned nucleosomes as specific bands in low ionic strength polyacrylamide gels. A temperature dependent redistribution of these nucleoprotein bands at low ionic strength (0.5×TBE and even 10 mM Tris−HCl, 0.2 mM EDTA, pH 7.4) was observed. This redistribution was attributed to the mobility of histone octamers between the different possible positions which, in this case, were part of a single rotational cluster, with the DNA coil in a conserved rotational orientation and the octamers moving within this coil.

Temperature dependence of octamer mobility on 5S rDNA

In our previous study of nucleosome mobility (Pennings *et al.*, 1991) we excised mononucleosomes from chromatin assembled on the tandemly repeated nucleosome positioning sequence 207_{18}. In this study, we assembled nucleosomes on the 207 bp DNA monomer and observed the same three mononucleosome bands on a nucleoprotein particle gel as were found in the earlier work (Figure 2, lane 1). We have proposed that the positions of the histone octamer on the DNA determine the migration of nucleosomes in a nucleoprotein gel in a manner analogous to the way the position of a bend in a DNA fragment is linked to its retardation in polyacrylamide gel (Wu and Crothers, 1984). Mononucleosomes in which the histone octamer is located at the ends of the DNA (positions 0 and 60) migrate more quickly than dominant position 10 (middle band), whereas nucleosome positions 20 and 30 near the middle of the fragment are found in the most slowly migrating band (Figure 2, lane 1). More evidence for this behavior will be discussed in the next section.

Strips containing the three mononucleosome bands were excised from the polyacrylamide gel and incubated in parallel at 4, 12, 17, 22, 27, 32 and 37°C in 0.5×TBE for 1 h. They were then mounted side by side on a second polyacrylamide gel and run under the same condition as in the first dimension, at 4°C in 0.5×TBE (Figure 1). In the control, kept at 4°C between the electrophoretic runs, only the three bands are found on a diagonal line, showing that there was no redistribution of the octamer positions at 4°C. Incubations at increasing temperatures result in the gradual emergence of a pattern of nine dots, resulting from the redistribution of each of the three bands into three bands characteristic of the positions of the octamers on the 207 bp DNA (Pennings *et al.*, 1991). There is a preference noticed for the most quickly migrating complement or end positions. Moreover, the original lower band of end positions only starts redistributing at the highest temperature. Positions 20 and 30 (lying around the middle of the 207 bp fragment and migrating most slowly in polyacrylamide gel, Figure 2, lane 1) readily redistribute to the dominant position 10 (middle band), and in the experiment shown, the upper band yields a faint middle dot even at the 4°C incubation. There may be a hint of an upstream directionality here as will be discussed below. Downstream mobility does exist, however, since a slower derivative of the middle band emerges at 27°C in parallel with an equally intense faster dot, i.e. an octamer at position 10 can move downstream to position 20 or even 30 as readily as it can move upstream to end position 0.

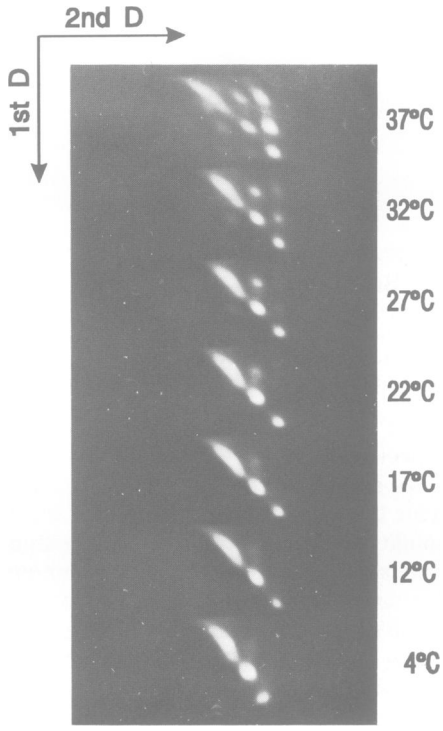

Fig. 1. Effect of temperature on mobility of octamers on 207 bp 5S rDNA. Nucleosomes in nucleoprotein gels migrate as three well defined bands (e.g. see Figure 2, lane 1). These bands were excised from the first dimensional gel. Identical gel strips were incubated at different temperatures, as indicated, between the first and the second dimension electrophoresis, both run at 4°C in 0.5×TBE.

414 bp 5S rDNA mononucleosome mobility

To investigate further the nature of histone octamer mobility at low ionic strength we constructed a head-to-tail dimer of 207 bp of 5S rDNA to give a 414 bp DNA molecule. Predominantly single nucleosome particles were assembled on this 414 bp fragment at low octamer:DNA ratios. The aim of the experiment was to study both short range and long range mobilities of nucleosome particles. Short range mobility would show up as redistributions of the nucleosome particles within the 207 bp monomer units of the dimer whereas long range mobility would result in the nucleosome migrating from one monomer to the other. As can be seen in Figure 2, lane 2, there was a dramatic increase in the complexity of the polyacrylamide gel banding pattern for the 414 bp mononucleosome compared with the 207 bp mononucleosome (lane 1). Also shown in Figure 2 are the assignments of the bands to the different positions of the nucleosome on the 414 bp DNA. These assignments will be discussed along with the data of Figure 3 and are a verification of the proposed link between electrophoretic retardation and the position of a histone octamer relative to the middle of the DNA fragment.

By labeling one end of the 207 bp dimer specifically, an assignment of a positioned nucleosome to one or other of the two halves should be feasible following an *AvaI* restriction digestion to separate the monomeric halves of the fragment after reconstitution. This is because the familiar 3-band pattern of the 207 bp particles is to be expected in polyacrylamide gels. For example, a nucleosome in the dominant position might migrate differently in nucleoprotein gel depending on whether it lies on the right or the left half

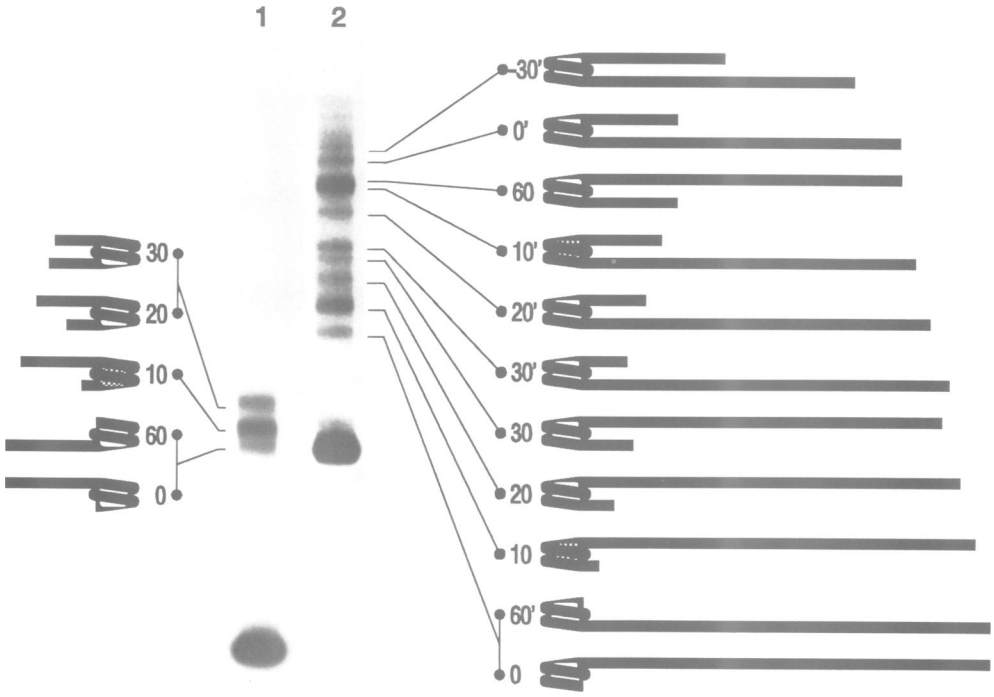

Fig. 2. Electrophoretic migration and position of mononucleosomes. Gel electrophoresis of reconstitutions with 207 bp and (dimer) 414 bp 5S rDNA (lanes 1 and 2 respectively) end labeled by Klenow filling in with [α-^{32}P]dCTP. On each side are drawings of the proposed positioned mononucleosomes that give rise to the observed bands (see text and Figure 3). Numbers refer to positioned nucleosomes. Protruding DNA and nucleosome dimensions are drawn to scale, as determined by micrococcal nuclease defined core particle boundaries (Meersseman *et al.*, 1991; see Figure 3C). Shaded octamers represent the dominant position.

of the 414 bp dimer, but complete cutting with *Ava*I should produce 207 bp of free DNA and the middle band of the 207 bp mononucleosome only. Because just one end of the 414 bp fragment would be labeled, we would be able to deduce which half of the dimer the nucleosome had been on. Furthermore, we can determine whether an additional incubation step at 37°C prior to the *Ava*I digest causes the redistribution of nucleosome particles from positions in one half of the dimer to positions in the other half.

Mononucleosomes were assembled on the 414 bp DNA dimer, which had been radioactively labeled on either end. These two labeled 414 bp mononucleosomes were run side by side on a 5% polyacrylamide gel in 0.5×TBE at 4°C. Both lanes were excised and split lengthwise. One half gel strip of each was left at 4°C while their complements were incubated at 37°C to allow for redistribution of the positioned nucleosomes. We subsequently equilibrated all strips against 2 mM $MgCl_2$, 10 mM NaCl, 40 mM Tris−HCl (pH 8.0) at 4°C and incubated them later with *Ava*I. Note that 2 mM $MgCl_2$ inhibits octamer mobility (Pennings *et al.*, 1991) during the restriction digestion. The strips were then equilibrated against 0.5×TBE at 4°C and electrophoresed in the same conditions on a second dimension polyacrylamide gel (Figure 3).

As expected, a control gel strip run in the second dimension without incubation at an elevated temperature and without digestion with *Ava*I features the first dimension pattern of Figure 2, lane 2 on a diagonal line (not shown). On digestion with *Ava*I the 414 bp nucleosomes are cut into 207 bp nucleosomes and free 207 bp DNA. In this two-dimensional gel analysis, the 207 bp mononucleosomes migrate in three columns of dots representing a sideways view of the upper, middle and lower bands of these mononucleosomes (Figure 2, lane 1). This pattern of bands is indicated by the rectangles (Figure 3). The rectangles of dots in Figure 3A ensue from 207 bp mononucleosomes located on the first half of the 414 bp dimer, whereas the rectangles in Figure 3B come from nucleosome particles located on the second half of the dimer (indicated in Figure 3C by the primed numbers). The dots or smears situated under each rectangle come from the trailing ends of the large amount of unreconstituted, partially undigested DNA, which was cut from the gel strips to avoid overexposed blobs on the autoradiographs. Material located to the left of each rectangle is from uncut 414 bp nucleosomes whereas cut free 207 bp DNA monomers migrate on the DNA column just to the right of each rectangle. We will verify whether proximity of a nucleosome position to the end of the 414 bp DNA fragment is proportional to the electrophoretic velocity of the particle, looking from both ends of the DNA fragment: the left end in rectangle 1 and the right end in rectangle 2. Analysis of the dots in the rectangles of Figure 3 will be made with reference to the corresponding band in the first dimension electrophoresis of 414 bp mononucleosomes (Figure 2, lane 2). Some unexpected redistribution is visible for rectangle 1, but not to the extent that the 'unredistributed' pattern is concealed.

Upon *Ava*I digestion, the most rapidly migrating 414 bp mononucleosome band yields the most rapidly migrating dots (or end positions) in the second dimension in both rectangle 1 and rectangle 2. The dot in rectangle 1 most probably comes from the mononucleosome located at the upstream end of the dimer, i.e. position 0 of Figure 3C. The dot in rectangle 2 then comes from position 60′, the downstream end of the dimer. The second 414 bp mononucleosome band,

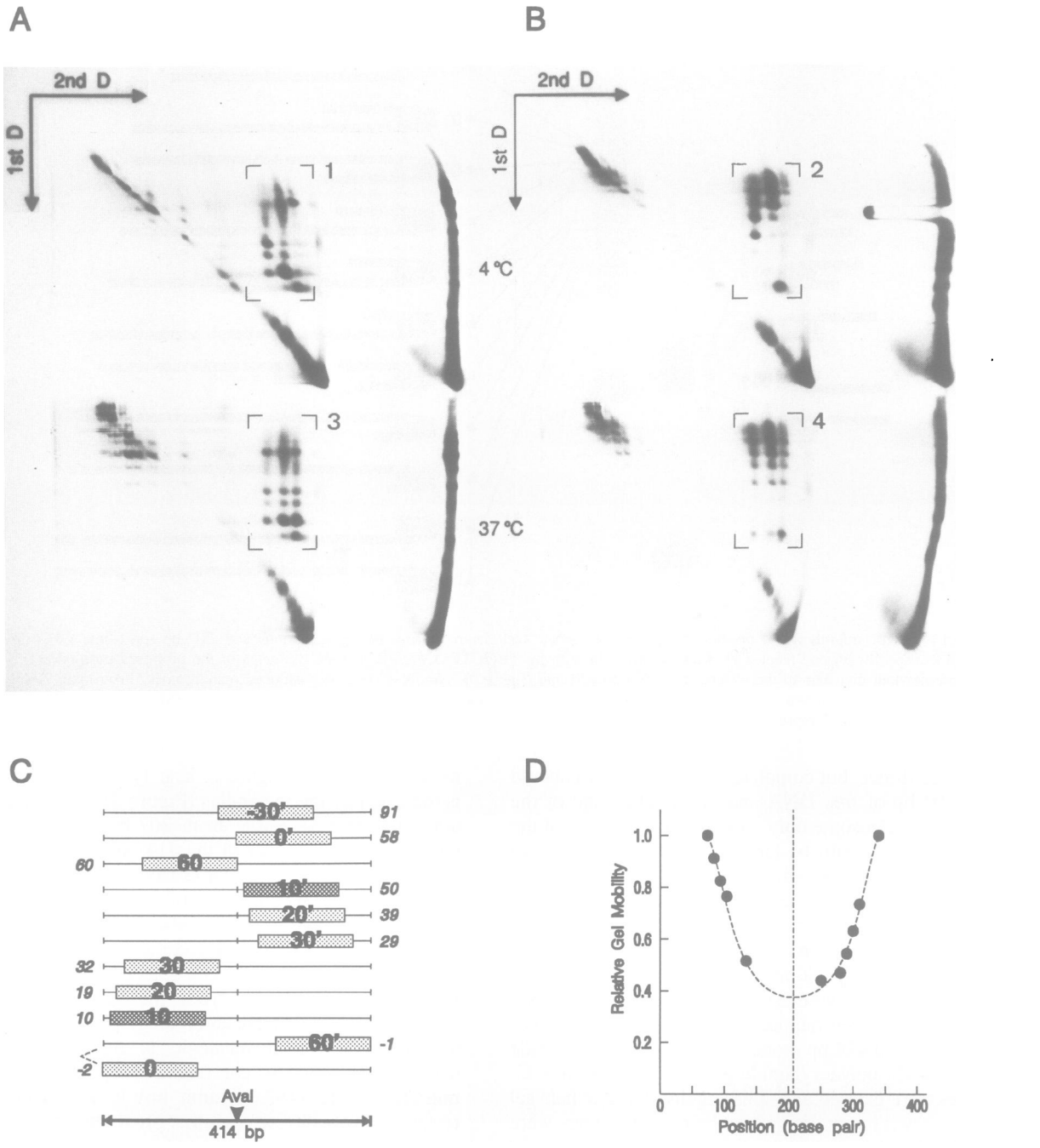

Fig. 3. Identification of positioned 414 bp 5S rDNA mononucleosomes and their mobilities. 414 bp 5S rDNA nucleosomes labeled by filling in the left 3′ end using [α-^{35}S]dCTP (**A**) or the right 3′ end using [α-^{35}S]dATP (**B**). First dimension gel electrophoresis was followed by a temperature incubation at 37°C or 4°C and subsequent *Ava*I digestion in the gel strip. Second dimension electrophoresis was, as in the first, at 4°C in 0.5×TBE. Highlighted rectangles represent 207 bp nucleosomal digestion products derived from 414 bp positioned nucleosomes, separated as in Figure 2, lane 2. **C**: Schematic representation of 414 bp 5S rDNA mononucleosome positions according to their electrophoretic mobilities. Bold numbers refer to positioned nucleosomes as in text. Italic numbers represent the length (in base pairs) of the shortest DNA end, protruding from the nucleosome, as determined in Meersseman *et al.* (1991). Shaded bars represent the dominant position. **D**: Correlation between electrophoretic mobility and nucleosome position (taken from midpoint of core particle).

following *Ava*I digestion, gives rise to a strong middle dot in rectangle 1 and no dots in rectangle 2. The dot in rectangle 1, a 207 bp mononucleosome corresponding to the dominant position 10 in Figure 2, lane 1, is identified as coming from (dominant) position 10 of a 414 bp mononucleosome (Figure 3C). The third band of 414 bp mononucleosome yields in the second dimension the most slowly migrating and middle dots in rectangle 1 and no dots in rectangle 2. It is probably from a mononucleosome located at position 20, only if followed by position 30 in the next band. The middle dot is probably due to redistribution, in view of the easy transition from positions 20 and 30 to position 10 discussed previously for the 207 bp mononucleosome. The fourth band shows the most slowly migrating dot in rectangle 1 and no dots in rectangle 2 and can indeed be identified as position 30. The fifth band, which lies very close to the

fourth, shows nothing in rectangle 1 and the most slowly migrating dot in rectangle 2 and probably represents position 30′ if followed by position 20′ in the next band. The sixth band yields nothing in rectangle 1 and the most slowly migrating dot and a minor middle dot in rectangle 2. Assuming that the previous band contains position 30′, this band is indeed identified as position 20′ with some redistribution into position 10′ as discussed above for the left half. From this point on there seems to be increasing interference with dots derived from dinucleosomal bands, which are specifically enhanced with increasing octamer:DNA ratios (not shown). Rectangle 1 shows smears in the most slowly migrating and middle regions and one prominent most rapidly migrating dot in the area where position 60 is expected, and it is to this position that we tentatively link the eighth band of the 414 bp mononucleosome. Rectangle 2 shows three columns consisting of most slowly migrating dots and mainly middle dots, and only the most rapidly migrating dots in the uppermost level. The (strong) seventh band is therefore identified as (dominant) position 10′. From Figure 2, lane 2 it is clear that the bands for positions 10′ and 60 overlap partly into a slightly broader band. The eighth band does resolve in weaker exposures. We are unable to make firm band assignments for positions 0′ and −30′ since their *Ava*I sites are protected and/or there is too much dinucleosomal noise. We do feel, however, that the presented data make a strong case for a correlation between position and electrophoretic migration of nucleosomes in polyacrylamide gel as is summarized graphically in Figure 3D.

Rectangles 3 and 4 provide patterns for 414 bp DNA mononucleosomes that were incubated at 37°C in 0.5×TBE prior to the *Ava*I digestion. They should reveal whether there is nucleosome mobility from one 5S rDNA positioning cluster to the other on 5S rDNA dimers. Rectangle 4 still shows a gap between what was the *Ava*I digestion product of positions 60′ and 30′ in rectangle 2. Any mobility from the left half of the DNA dimer to the right half would have generated nucleosomes with their octamers on the right, labeled half in those first dimension bands that contain positions 10, 20 and 30 of the 414 bp mononucleosome. Since there is no significant amount of radioactive label in the area where these positions should deposit their 207 bp nucleosomal halves, we can conclude that mobility from the left cluster of positions to the right one has not taken place. For cluster to cluster octamer mobility in the opposite direction there is a more complex pattern in rectangle 3 to interpret. Newly generated dots on the ordinates of positions 10′ and 0′ would be obscured by dinucleosomes and position 60′ coincides with position 0. Only position 30′, which lies close to position 30, and position 20′, which may be partly obscured by the most slowly migrating and middle smears from rectangle 1, could potentially be observed after redistribution from the right half of the dimer to the left, labeled half. There is, however, not even a hint of additional radioactively labeled dots on either the 30′ nor the 20′ ordinate in rectangle 3. We therefore conclude that there is no evidence for redistribution of positioned nucleosomes between either cluster of positions in this experiment.

Having assigned most of the mononucleosome bands, we were able to interpret the effect of 37°C incubation on these positioned nucleosomes omitting the restriction digest. A polyacrylamide strip containing 414 bp mononucleosomes

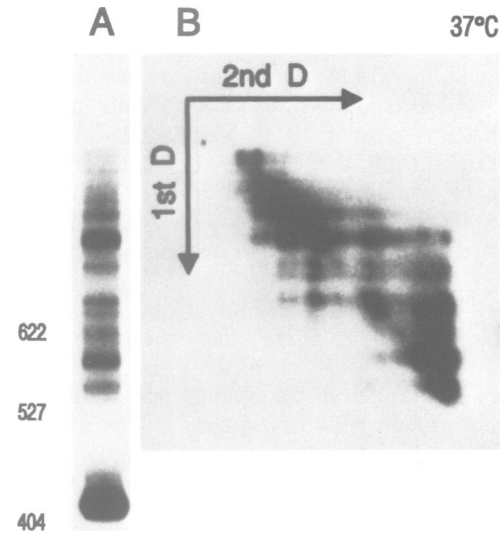

Fig. 4. Mobility of histone octamers on 414 bp 5S rDNA nucleosomes. Gel strip containing nucleosomes was incubated at 37°C and run in the second dimension (**B**) in the same conditions as the first (**A**), at 4°C in 0.5×TBE. End labeling was by Klenow filling in with [α-^{32}P]dCTP. Lengths are in bp.

was incubated at 37°C in 0.5×TBE and run in the second dimension at 4°C in 0.5×TBE. The redistribution of this complex pattern of bands created by nucleosomes positioned on the dimer 5S rDNA is evident by a considerable deviation from the diagonal line in the second dimension electrophoresis (Figure 4). The lattice-like appearance of the redistribution pattern indicates that particular nucleosome positions convert to other positions. In agreement with the *Ava*I restriction position analysis above, however, nucleosomes clearly cannot move freely to any position, which would give a uniform square of dots. Instead, three distinct regions are observed in the redistribution pattern. Crudely, the lower triangular part of the pattern represents redistribution among the positions clustered mostly on the left half of the dimer sequence, the middle rectangular part shows redistribution in the cluster mostly on the right half of the dimer. The dots in the upper left part of the figure are specifically enhanced with increasing octamer:DNA ratios (not shown) and probably correspond to fragments carrying two octamers, as discussed above. First, a nearly fully formed square of dots results from the redistribution on the right half of the dimer fragment, as opposed to a more triangular area for the cluster on the left half of the DNA. This suggests that nucleosomes positioned on the left half of the dimer get trapped in the left end position (most rapidly migrating row of dots). This is also shown in the restriction digestion pattern of the 414 bp nucleosome following incubation at 37°C (Figure 3, rectangle 3): there is a lack of redistribution in the lowest row of dots derived from position 0, as opposed to the lowest row of dots in rectangle 4 showing redistributions from position 60′. In keeping with the redistribution data of the position analysis above, the absence of dots below the middle of the pattern, to the left of the lower triangular pattern, shows that nucleosomes originally positioned in the left cluster cannot reach positions in the right cluster. Mobility from the right cluster to the left would be witnessed by dots to the right in the middle

of the pattern. However, the presence of the faster dots is not conclusive because of overlap with right end position 60′ and with redistribution products from position 60. If the second fastest column of dots represents nucleosomes redistributed to position 10, then one weak dot could point to migration from the right to the left. This possible discrepancy with the restriction analysis above could be explained if these particular nucleosomes were not adequately represented due to dissociation or inaccessibility to restriction enzyme.

Studies on nucleosome mobility require techniques that can track nucleosomes as they move from one position to another, since the net result of the redistributions is most often not different from the original position distribution. The nucleoprotein gel analysis used in this study has the unique quality of separating differently positioned nucleosomes in the native state. However, because of the type of manipulations involved, the approach is not well suited for studies in which different parameters are systematically varied, nor for time course experiments due to inefficient heat exchange with the gel strips. To complement and control the nucleoprotein gel assay, we have extended the nucleosome mobility studies to solution, using constructs of nucleosomes ligated to naked, labeled DNA. Following incubation at higher temperature in low ionic strength buffers, we determined if the nucleosomes had moved onto the labeled DNA. 207 bp 5S rDNA nucleosomes, blocked by filling in with Klenow at their left end, were ligated at the *Ava*I site to 207 bp DNA fragments labeled at the right 3′ end. Incidentally, addition of the extra DNA converts the three bands corresponding to the end positions, position 10 and positions 20 and 30, to five bands that comigrate with the corresponding left half nucleosome positions from reconstitutions on dimer DNA (not shown). The redistribution incubations were followed by restriction digestion and nucleoprotein gel electrophoresis to reveal the presence of new radiolabel in the nucleosome fraction. In accord with results from the experiments described above, we detected no migration of nucleosomes onto the right DNA fragment. Unfortunately, ligation of the inverse construct, with left end labeled DNA to the left end of nucleosomes (with a blocked right end), suffered from wrong end ligations. Similar difficulties, compounded with the occurrence of single strand ligation, were encountered in experiments requiring blunt end ligation of bulk nucleosomes to the 5S rDNA sequence or blunt end ligation of 5S rDNA nucleosomes to 5S rDNA, trimmed at one end with Bal31. This method seems therefore to be of limited use for studying short range nucleosome mobility.

Mobility of bulk mononucleosomes

How general is the described nucleosome mobility? Is it specific for certain sequences or is it a general aspect of H1 depleted chromatin? We verified this using chicken mononucleosomes. They were prepared from long H1 depleted chromatin using restriction enzymes to avoid trimming of the protruding DNA ends. This was necessary since differentiation in electrophoretic migration of mononucleosomes requires particles containing more than ~170 bp of DNA (Meersseman *et al.*, 1991). A second dimension electrophoresis in deproteinizing conditions, revealing the DNA lengths in nucleosomes with the same mobility, illustrates this further (Figure 5B). The DNA fans out in the second dimension starting at ~190 bp.

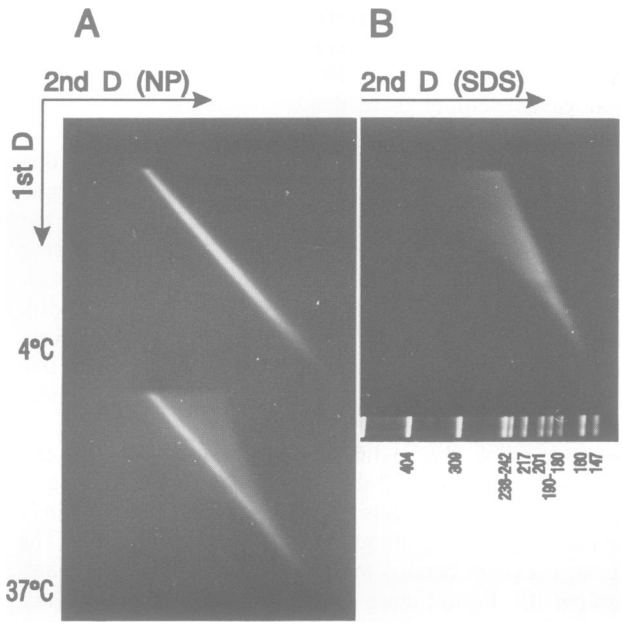

Fig. 5. Mobility of bulk H1 depleted nucleosomes. **A**: Gel strips containing mononucleosomes were excised and incubated at 37°C or 4°C (control) and run in the second nucleoprotein (NP) dimension as in the first, at 4°C in 0.5×TBE. **B**: An identical gel strip was soaked in SDS and run in the second dimension on a DNA gel in TBE, 0.1% SDS. Marker is a pBR322 *Msp*I digest.

Mononucleosomes that are electrophoretically slowest contain DNA lengths ranging from ~220 bp to >300 bp. The experiment investigating low ionic strength octamer mobility involved a second dimension electrophoresis in 0.5×TBE at 4°C, as in the first dimension. The control incubation at 4°C shows a diagonal line, whereas the incubation at 37°C clearly causes many mononucleosomes to alter their electrophoretic behavior (Figure 5A). There is a marked bias for an increase in electrophoretic velocity indicating preference for nucleosome particles to occupy end positions. A significant number of nucleosomes, however, are not mobile, as illustrated by the fairly strong diagonal line that is still present for the 37°C incubation. These results indicate that low ionic strength mobility of histone octamers is potentially a general phenomenon but not on all DNA sequences.

Mobility of nucleosomes reconstituted onto Alu family repeats

To understand this mobility of octamers better, we studied the behavior of nucleosome particles on another single DNA sequence of a totally different nature and genomic environment from 5S rDNA. Representatives of the Alu family of interspersed repetitive elements seemed good candidates because of their general presence throughout the human genome and because they provide for the nucleosomal DNA length requirements. The Alu fragments or Bam linked ubiquitous repeats (BLURs) originate from renatured and S1 treated human DNA. They are G+C rich and are composed of two homologous portions arranged in a head-to-tail dimer and with an oligo(A) or A-rich region at the right end of each half of the dimer. We reconstituted Alu fragments BLUR2 and BLUR11 (Deininger *et al.*, 1981), 302 bp and 279 bp in length, respectively, with HeLa octamers and ran the reconstitutes on nucleoprotein gel

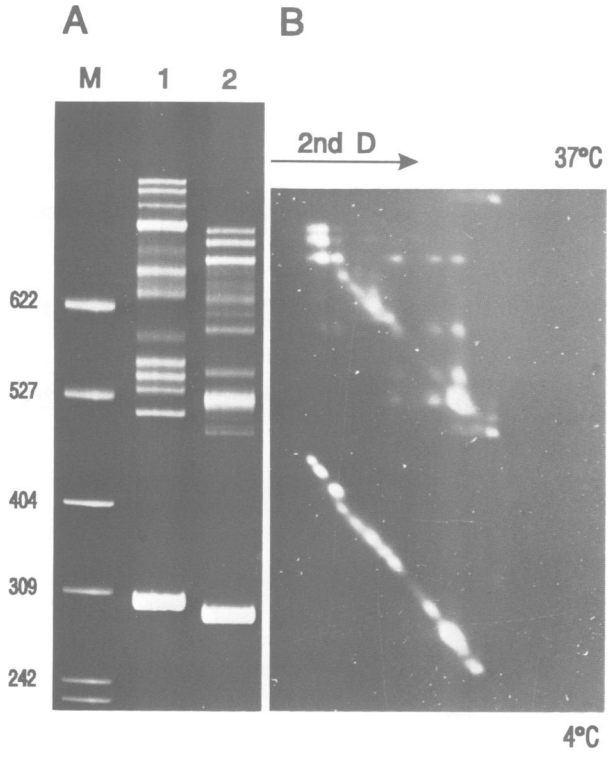

Fig. 6. Mobility of nucleosomes reconstituted on Alu family repeats. **A:** Gel electrophoresis of reconstitutions with BLUR2 (lane 1) and BLUR11 (lane 2). Marker is a pBR322 *Msp*I digest. **B**: Gel strips containing BLUR11 nucleosomes and incubated at 37°C and 4°C (control), were run in the second dimension in the same conditions as the first, at 4°C in 0.5×TBE.

(Figure 6). Both reconstitutes give several bands of different intensities in ethidium bromide stain. This would again be indicative of a series of positioned nucleosomes on these DNA fragments. A second dimension run in 0.5×TBE at 4°C of a gel strip containing BLUR11 nucleosomes shows a diagonal line of this multitude of bands for the 4°C control. Incubation at 37°C between the electrophoretic runs redistributes these dots into a pattern similar to that observed for the 414 bp 5S rDNA mononucleosomes but differing in a few important aspects. Although there is a slight bias for more quickly migrating dots, the redistribution does not result in nucleosomes positioned at the extreme ends of the DNA, as evidenced by the absence of very intense dots in the column of most rapidly migrating dots. Only the lower right corner of the pattern contains a limited set of redistributing end positions with no redistribution outside this particular cluster. This would argue for a small set of possible positions for nucleosomes reassembled on or near one end of the fragment, probably the left end. The right end of the BLUR11 sequence has a poly[d(A)·(dT)] stretch of 19 bp and a *Bam*HI end. This poly[d(A)·(dT)] tail may be less preferred for assembly into nucleosome particles. The strongest band of the 1D pattern could represent the rightmost possible 'end position'. This right side 'end position' seems to trap rearranging nucleosomes during redistribution not unlike the 0 position for 414 bp 5S rDNA mononucleosome and probably the 0 position for the 207 bp 5S rDNA nucleosome as well. We observe four dotted horizontal lines: the two lowest starting with the right 'end position' ordinate and the one right above it, showing preferential redistribution into that particular 'end position'. The upper two horizontal lines contain dots with similar electrophoretic velocities which seem to redistribute readily into each other and show a slight preference for the column of the right 'end position'. Another cluster of dots, lying between the two upper horizontal lines, does not participate in this prevailing pattern of redistribution. It seems to consist of nucleosomes that are hardly mobile in their particular location. We do not think they are dinucleosomes, since those are expected to be electrophoretically slower (we assume that the two uppermost bands contain dinucleosomes). In summary, the redistribution of bands for the BLUR11 reconstitutes shows that mobility occurs readily on this sequence for nucleosomes migrating in the four horizontal lines. The pattern also reveals that some nucleosomes containing this very same sequence show limited mobility or none at all, as a consequence of the starting position of that nucleosome.

This redistribution behavior for BLUR11 mononucleosomes complements the data obtained for bulk chicken erythrocyte mononucleosomes. Together they suggest that low ionic strength mobility of histone octamers is potentially a general phenomenon that does not apply to all DNA sequences and/or depends on the starting position of the nucleosome.

Discussion

The two-dimensional gel patterns provide convincing evidence for a dynamic behavior of a major subset of nucleosomes. We demonstrate temperature dependent nucleosome mobility for three systems, going from the very specific example of nucleosomes assembled on different 5S rDNA constructs, over nucleosomes assembled on Alu family repeats, to the general case of bulk nucleosomes. We show that the gel mobility assay is a sensitive tool for the analysis of nucleosome positioning and its dynamics. The technique was applied to gain information on systems with increasing complexities, the detailed information obtained on the 5S rDNA system aiding in interpreting the patterns.

Multiple positions of mononucleosomes carrying at least 190 bp of DNA can be resolved in nucleoprotein gels. Electrophoretic mobility of such nucleosomes is a function of the proximity of the nucleosome position to the center of the DNA. As proposed earlier (Pennings *et al.*, 1991), nucleosome positioning affects gel electrophoretic mobility of nucleosomes in much the same way that the location of a bend in a DNA fragment is linked to its retardation in polyacrylamide gels (Wu and Crothers, 1984). By analogy, this effect on retardation could be an indicator of the angle formed between the pieces of DNA exiting from the core particle.

The sea urchin 5S rRNA gene sequence contains a strong nucleosome positioning site (Simpson and Stafford, 1983; Simpson *et al.*, 1985). On this site, nucleosomes reconstitute in a dominant position flanked by minor positions, all with identical rotational setting of the DNA coil (Meersseman *et al.*, 1991; Dong *et al.*, 1990). Histone octamers shift between positions in this cluster at 37°C in low salt conditions (Pennings *et al.*, 1991). The interaction between DNA sequence and histone octamer alone is at the basis of this behavior. It is nevertheless clear that DNA end effects can arise on shorter fragments.

It is confirmed that low salt short range sliding is a temperature dependent process, with nucleosomes moving

further with increasing temperature. There are, however, boundaries to this mobility, because nucleosomes do not travel outside the cluster of possible positions to the next cluster. The translational differences between the possible nucleosome positions on 5S rDNA span only nine helical turns (Meersseman *et al.*, 1991) and mobility of histone octamers on this sequence is limited to this cluster of positions around a dominant position.

Importantly, this dynamic aspect of nucleosomes is not unique to 5S rDNA and appears to be a general behavior. We have shown that nucleosome mobility is observed with native bulk H1 depleted nucleosomes. A significant proportion of the bulk nucleosomes, however, does not display any movement at 37°C. This interesting point is further illustrated with nucleosomes positioned on Alu family repeats. Here also, a single nucleosome can assemble in any of several partly overlapping sites. Some positions revealed limited or no redistribution, whereas others were part of redistributing sets of nucleosome positions. This demonstrates that one sequence can host both types of positioning.

An important determinant in the positioning of nucleosomes is the DNA anisotropy of flexibility required to accommodate tight bending around the nucleosome (Satchwell *et al.*, 1986). Because the binding affinity is the cumulative result of the small contributions of many bends, positioning is often rotationally unique but translationally degenerate (Shrader and Crothers, 1990). There are several examples in the literature of multiple positions spaced by 10 bp (Lowman and Bina, 1990; reviewed by Simpson, 1991). Whereas small translational variations on position with the same rotational setting of the DNA around the octamer differ little in binding affinity, the accommodation of a DNA sequence with non-compatible bendability holds a larger energy penalty (Shrader and Crothers, 1990). The mobility of a nucleosome is likely to depend on the sequences flanking its position. A nucleosome would be allowed to move as long as the coiling path of the DNA could be continued beyond its immediate location. Two overlapping nucleosome positions with different rotational settings of the DNA around the histone octamer may theoretically not both satisfy this condition.

Nucleosomes in motion may be visualized as following a corkscrew movement within the superhelical path of the DNA. Mobility may be limited by the same elements that act as boundaries to nucleosome positioning (reviewed by Simpson, 1991). We think that here could lie the difference between the short range mobilities of nucleosomes at low salt and the *in vitro* observation of high salt long range nucleosome sliding. The non-physiological ionic conditions required for this seemingly related process would be expected to reduce interactions between the histones and DNA such that any barriers to long range sliding would no longer be effective.

The generality of short range nucleosome mobility provides a compelling argument that the position of a nucleosome should be regarded as a probability rather than a static factor-type of binding. This means that possible positions of nucleosomes can be precisely defined but at the same time that the actual locations are dynamic. Consequently, chromatin may be a more dynamic structure than is generally assumed.

In vivo, local ionic conditions differ and a number of other factors, such as H1, histone modifications, DNA binding proteins or interactions with neighboring nucleosomes, may assert their effects. Nucleosomes may be immobilized by any of these factors. If, as expected, nucleosome mobility occurs *in vivo*, then this has implications not only for the way we view nucleosome positioning but also for the way nucleosomes are thought to be involved in active processes. An interesting possibility is that potentially mobile nucleosomes could be fixed or free to move depending on functional requirements. This would provide a possibility for adaptive response by nucleosome reorganization. Binding sites for *trans*-acting factors could become exposed. Binding of a protein factor to the DNA could confine the nucleosome to a less preferred position (with lesser affinity), which could aid destabilization. These and other possibilities could be tested experimentally.

Our results support a more dynamic view of the chromatin structural organization. This seems essential in order to understand how chromatin structure can be a functional component of active nuclei.

Materials and methods

Preparation of DNA substrates

Monomer 207 bp 5S rDNA fragments were generated from the tandemly repeated insert of plasmid p5S207-18 (gift from Dr R.Simpson) by *Ava*I restriction digestion. Head-to-tail dimers of this sequence were obtained by ligation at the asymmetric *Ava*I site (CCCGAG) with T4 DNA ligase, starting from two populations of monomer fragments that were each partly filled in at one 3′ end (either with dTTP or with dCTP and dGTP) using Klenow fragment, thus minimizing polymerization during ligation. The resulting dimers could be radiolabeled at the right (3′ end) by filling in with [α-^{35}S]dATP or at the left end with [α-^{35}S]dCTP, adding the respective dNTPs to enhance selectivity. Alu family DNA fragments of 302 bp and 279 bp were excised with *Bam*HI from plasmids pBLUR2 and pBLUR11 respectively (gifts from Dr C.Schmid). DNA fragments of interest in the above procedures were purified on a Mono Q column (Pharmacia), eluting with a 0.7–0.8 M NaCl gradient in 0.2 mM EDTA, 20 mM Tris–HCl (pH 8.0).

Preparation of HeLa chromatin and histone octamers

HeLa S_3 cells were grown and nuclei were prepared as described by Yau *et al.* (1982) except that 10 mM NaCl, 3 mM $MgCl_2$, 250 mM sucrose, 0.1 mM PMSF, 10 mM Tris–HCl (pH 7.4) was used as the nuclei isolation buffer. Nuclei were digested with micrococcal nuclease (20 U/mg DNA, Cooper Biomedical) in the same buffer adding 1 mM $CaCl_2$. They were lysed by overnight dialysis against 10 mM NaCl, 0.2 mM EDTA, 0.1 mM PMSF, 10 mM Tris–HCl (pH 7.4) at 4°C. Histone octamers were obtained from long HeLa chromatin as in Meersseman *et al.* (1991).

Nucleosome reconstitution procedure

Reconstitutions were carried out at 7–10°C. HeLa histone octamers and 207 bp DNA substrate were mixed in a 0.9:1 (w/w) ratio to a final A_{260} between 2 and 5, in 2 M NaCl, 0.2 mM EDTA, 10 mM Tris–HCl (pH 7.4). To limit the assembly to a single nucleosome per DNA fragment, we used low octamer:DNA ratios [0.15–0.4:1 (w/w)] for DNA substrates longer than nucleosomal length. Small volumes were dialyzed to decreasing NaCl concentrations in 1 h steps [2–1.5–1–0.75–0.5 M NaCl, each including 0.2 mM EDTA, 10 mM Tris–HCl (pH 7.4)], and a final overnight step to 10 mM NaCl, 0.2 mM EDTA, 10 mM Tris–HCl (pH 7.4). Ligation of naked DNA to nucleosomes and concomitant isolation procedures were largely according to Lorch *et al.* (1987).

Preparation of chicken erythrocyte mononucleosomes

Long chromatin was prepared from chicken erythrocyte nuclei as described for HeLa nuclei. The long chromatin was depleted of histones H1/H5 by centrifugation through a sucrose gradient containing 600 mM NaCl, 0.2 mM EDTA, 10 mM Tris–HCl (pH 7.4) at 4°C. The H1/H5 depleted chromatin was digested with restriction enzymes *Alu*I, *Hae*III and *Rsa*I in 10 mM NaCl, 2 mM $MgCl_2$, 10 mM Tris–HCl (pH 7.4) at 37°C for 1 h. Mononucleosomes were isolated by sucrose gradient centrifugation in 10 mM NaCl, 0.2 mM EDTA, 10 mM Tris–HCl (7.4) at 4°C.

Gel electrophoresis

5% polyacrylamide nucleoprotein gels were run in 45 mM Tris, 45 mM boric acid, 1.25 mM EDTA (pH 8.3) (0.5×TBE) at 4°C at a maximum of 10 V/cm for 1.5 mm thickness. Second dimension gel electrophoresis in deproteinizing conditions was performed in a 5% polyacrylamide gel in TBE, 0.1% SDS. The unstained gel strip was soaked in TBE, 0.1% SDS prior to the second dimension electrophoresis. In the two dimensional nucleoprotein gels of the position redistribution experiments, series of samples were applied in duplicate and electrophoresed in the first dimension in 0.5×TBE at 4°C. The part of the gel destined to be used to view the separation of nucleoprotein particles in the first dimension was removed and stained (or dried and autoradiographed). From the duplicate part of the gel, which was kept at 0–4°C, each lane was excised and cut in half lengthwise. One half strip of each lane was left at 4°C, while the other was sealed in a small plastic bag and immersed at 37°C (or other temperatures) for 1 h. Next, control and incubated gel strips were arranged side by side on top of a second nucleoprotein gel in the cold, and the second dimension nucleoprotein run was performed in the same conditions as the first one.

Additional *Ava*I digestion of nucleosomes was performed inside the gel strips prior to the second dimension electrophoresis. The gel strips were first transferred to individual tubes on ice and equilibrated against 2 mM $MgCl_2$, 10 mM NaCl, 40 mM Tris–HCl (pH 8.0) with several buffer changes. The strips were then placed side by side in a tube and completely covered with 3 ml of buffer containing 3000 U *Ava*I. Diffusion of enzyme into the gel strips was allowed for several hours at 4°C on a rotating wheel. Next, they were sealed in plastic bags and immersed at 37°C for a 1 h digestion. The gel strips were then washed several times in 0.5×TBE in tubes on ice, and second dimension nucleoprotein electrophoresis was performed as above.

Acknowledgments

We thank Dr R.Simpson for his gift of plasmid p5S207-18 and Dr C.Schmid for the BLUR plasmids. We thank Tim O'Neill, Peter Yau and Gary Schroth for discussions. This work was supported by DOE grant (DE-F903 88ER 60673) and NIH grant (GM 26901).

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Received on March 26, 1992