Atomic force microscopy sees nucleosome positioning and histone H1-induced compaction in reconstituted chromatin

Masa H. Sato[a], Kiyoe Ura[b], Ken I. Hohmura[a], Fuyuki Tokumasu[a], Shige H. Yoshimura[a], Fumio Hanaoka[b], Kunio Takeyasu[a,*]

[a] *Department of Natural Environmental Sciences, Faculty of Integrated Human Studies, Kyoto University, Yoshida-Nihonmatsu-cho, Sakyo-ku, Kyoto 606-8501, Japan*
[b] *Institute for Molecular and Cellular Biology, Osaka University, Yamada-oka, Suita, Osaka 565-0871, Japan*

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Abstract **We addressed the question of how nuclear histones and DNA interact and form a nucleosome structure by applying atomic force microscopy to an in vitro reconstituted chromatin system. The molecular images obtained by atomic force microscopy demonstrated that oligonucleosomes reconstituted with purified core histones and DNA yielded a 'beads on a string' structure with each nucleosome trapping 158 ± 27 bp DNA. When dinucleosomes were assembled on a DNA fragment containing two tandem repeats of the positioning sequence of the *Xenopus* 5S RNA gene, two nucleosomes were located around each positioning sequence. The spacing of the nucleosomes fluctuated in the absence of salt and the nucleosomes were stabilized around the range of the positioning signals in the presence of 50 mM NaCl. An addition of histone H1 to the system resulted in a tight compaction of the dinucleosomal structure.**

Key words: Atomic force microscopy; Dinucleosome; Chromatin structure; Nucleosome positioning; Histone H1; Nucleosome compaction

1. Introduction

In the nucleus of eukaryotic cells, DNA is packaged into chromatin whose basic unit is a nucleosome. The nucleosome core particle consists of 146 bp of DNA wrapped around an histone octamer (two molecules of each of H2A, H2B, H3 and H4). Packaging of DNA into chromatin affects the dynamic processes of DNA metabolism, including transcription, replication, recombination and repair [1–3]. For example, the position of nucleosomes influences the access of *trans*-acting factors to target DNA and, thus, controls the transcriptional activity of the gene. Therefore, an elucidation of structure-function relationships of DNA/protein interaction at the chromatin level is critical for the understanding of the regulatory events of DNA metabolism in the nucleus. Recently, to avoid the technical difficulty inherent to handling a complex native chromatin with a large molecular mass, a simple model system, reconstituted chromatin, has been developed [4]. In this report, employing this system together with a new simple one molecule imaging technique, atomic force microscopy (AFM) [5–9], we directly visualize the nucleosome organization in chromatin.

*Corresponding author. Fax: (81) (75) 753 6549.
E-mail: takeyasu@gaia.h.kyoto-u.ac.jp

2. Materials and methods

2.1. Purification of nucleosome core particles, histone octamers and linker histone H1

Histone octamers were prepared from the nuclei of HeLa cells as described by O'Neill et al. [10]. Histone H1 was prepared from chicken erythrocytes by means of 5% perchloric acid extraction of nuclei and acetone precipitation [11].

2.2. DNA templates and nucleosome reconstitution

Oligonucleosomes were reconstituted onto linearized pBluescript SK^+ (Stratagene) vector DNA using the salt dialysis method [12]. Purified core histones (6 μg) were mixed with the *Eco*RI-digested 3 kbp plasmid DNA (5 μg) in 2.0 M NaCl, 10 mM Tris-Cl (pH 7.5), 1 mM EDTA, 0.1 mM PMSF and 1 mM 2-mercaptoethanol at 4°C. The samples were dialyzed at 4°C against 10 mM Tris-Cl (pH 7.5), 1 mM EDTA, 0.1 mM PMSF and 1 mM 2-mercaptoethanol and varying concentrations of NaCl as follows: 2.0 M NaCl, 1 h; 1.5 M NaCl, 4 h; 1.0 M NaCl, 4 h; 0.75 M NaCl, 4 h. The final dialysis was performed overnight into 10 mM Tris-Cl (pH 7.5), 1 mM EDTA, 0.1 mM PMSF and 1 mM 2-mercaptoethanol at 4°C. In dinucleosome reconstitution, biotinylated DNA was generated by PCR using a 5′-unlabelled primer (5′-TCTAGAACTAGTGGATCCCC) and a 5′-biotinylated primer (5′-GTACCAAGCCTCTCGAGAAAAG). The 437 bp DNA template, with one end labelled with biotin, contains two 197 bp tandem repeats of a *Xenopus borealis* somatic 5S RNA gene and associated upstream sequences (−64 to +122 relative to the start site of transcription, +1) encompassing the entire nucleosome positioning element [10]. After dinucleosome reconstitution, streptavidin was added to the biotinylated dinucleosome at an equal molar ratio.

2.3. Linker histone incorporation into dinucleosomal template

The reconstituted nucleosome cores (1 μg of DNA) were incubated with 0.2 μg histone H1 in 20 μl of binding buffer containing 10 mM Tris-Cl (pH 8.0), 50 mM NaCl, 0.1 mM EDTA at 26°C for 20 min.

2.4. Sample preparation for AFM imaging

Unless otherwise indicated, reconstituted nucleosomes were fixed with 0.1% glutaraldehyde at 4°C overnight. After overnight fixation, dinucleosomes were applied onto spermidine (1 mM)-treated mica surface. After 3 min, the mica was gently washed with distilled water and dried under nitrogen gas. Imaging was done using a Nanoscope IIIa with a type E scanner (Digital Instrument, Santa Barbara, CA, USA) in air under a tapping mode at room temperature. The probe used was made of a single silicon crystal with a cantilever length of 129 μm and a spring constant of 33–62 N/m (Digital Instrument, Santa Barbara, CA, USA) that were used for imaging. Imaging was performed in the height mode with a scanning rate of 3–4 Hz and a driving amplitude of 40–80 mV. The images were captured in a 512×512 pixel format and the captured images were flattened and plain-fitted before analysis.

3. Results and discussion

3.1. AFM visualizes nucleosome positioning and compaction

Imaging by AFM does not require complicated specimen preparation procedures, including staining and shadowing,

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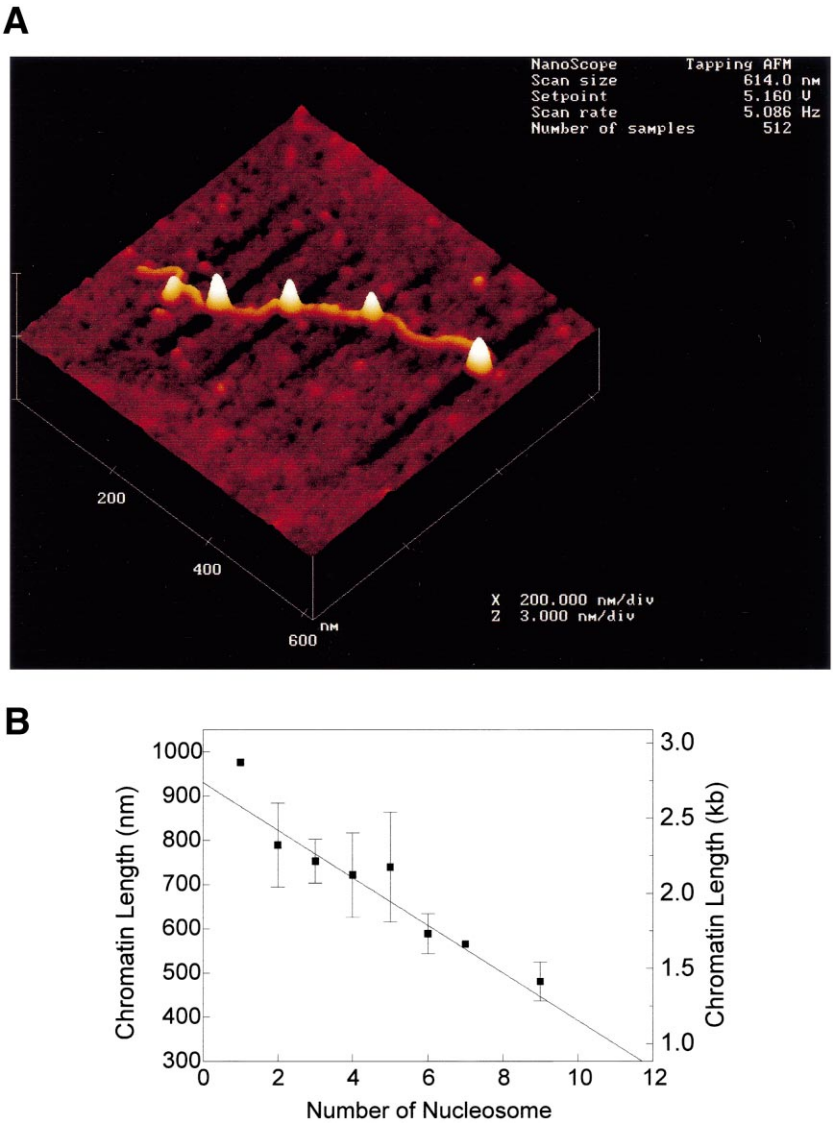

Fig. 1. (A) Typical AFM image of the chromatin structure reconstituted in vitro using linearized bacterial plasmid DNA as a template. Histone octamers were prepared from HeLa cell nuclei and chromatin was reconstituted as described in Section 2. The reconstituted chromatin preparation was applied onto a spermidine-treated mica surface without fixation and dried under nitrogen gas. Imaging was done using a Digital Instrument Nanoscope IIIa with a type E scanner in air under tapping mode at room temperature. (B) Linear relationship between the length of the reconstituted chromatin and the number of nucleosomes formed on the DNA. The length of chromatin strands with different numbers of nucleosomes formed was measured from one end to the other. The data were collected from 50 individual images and used for a calculation of the length of DNA.

yet, provides a molecular resolution. When we applied the AFM technique to the reconstituted chromatin, this technique detected a well-known beads on a string structure (Fig. 1A). The length of the reconstituted chromatin strand was gradually decreased in proportion to the number of nucleosomes formed on the template DNA, i.e. there was a linear relationship between the number of nucleosomes formed on the DNA and the length of the chromatin (measured from one end to the other). The degree of DNA shortening was calculated to be about 158 ± 27 bp per one nucleosome particle forming (Fig. 1B).

A powerful approach to the analysis of the structure-function relationship of chromatin has been the use of reconstituted oligonucleosomal systems that contain a set of signals for nucleosome positioning [9,12–17]. We made use of synthetic dinucleosomal templates containing two 197 bp tandem repeats of the 5S RNA gene and, to identify the direction of the DNA fragment, labelled the downstream end of the template gene with biotin followed by streptavidin conjugation (see the arrow head in Fig. 2A). Fig. 2A shows a typical image of a dinucleosome observed by AFM. Measured from the labelled downstream end, the proximal and the distal sites for nucleosome positioning were identified at positions around 30 nm and 60 nm, respectively (Fig. 2B). These positions determined by AFM imaging coincided with the sites for nucleosome positioning determined biochemically using the same dinucleosome preparation [12]. Thus, the AFM technique clearly showed that two nucleosomes were located within the respective positioning signals. The nucleosome positioning to the distal site, but not to the proximal site, rather fluctuated on the template DNA. These results support the previous implication on this system that the nucleosome at the prox-

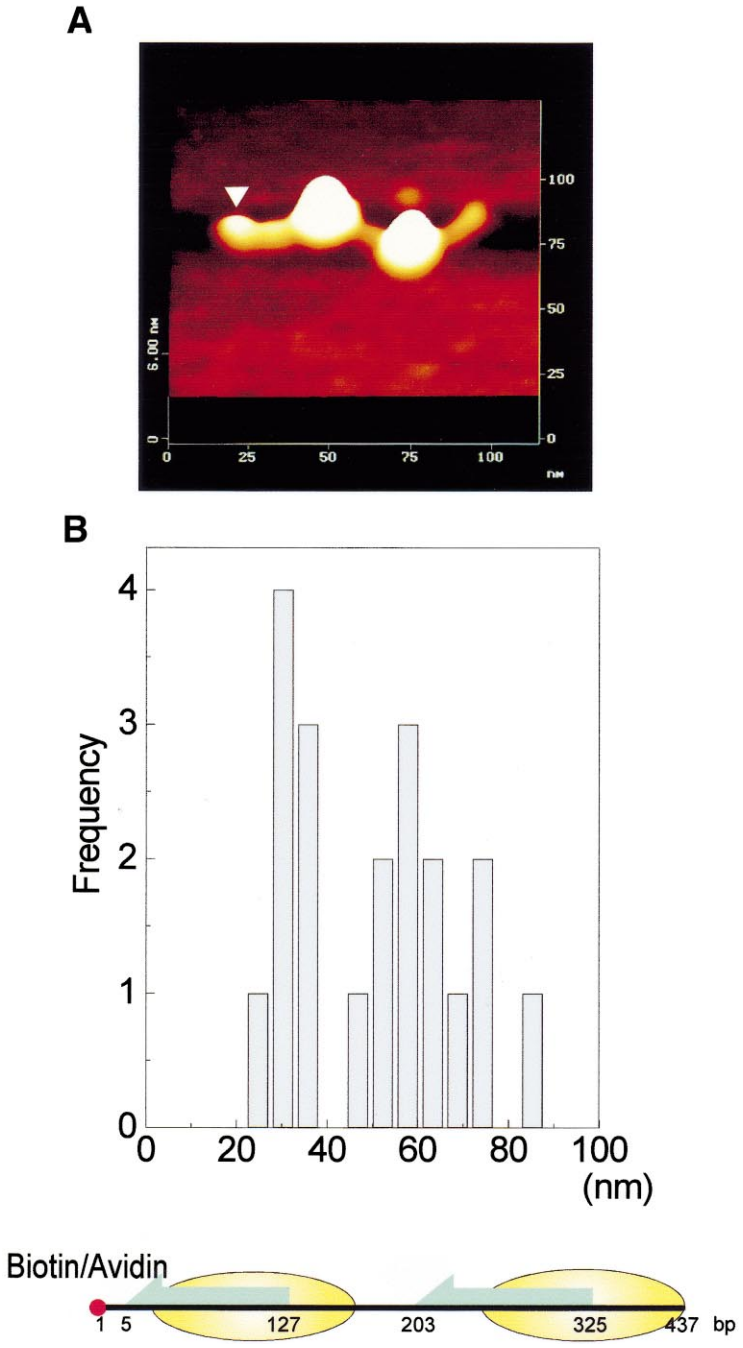

Fig. 2. (A) Typical AFM image of an in vitro reconstituted dinucleosome that shows nucleosome positioning. Biotinylated DNA was generated by PCR, reconstituted into a dinucleosome as described in Section 2 and labelled with streptavidin at an equal molar ratio. The arrow head shows the biotin-labelled end of DNA conjugated with streptavidin. (B) Frequency histogram of the distance from the labelled end to each nucleosome center. The number of observations was plotted against the observed position of the nucleosome (apparent length from the biotinylated end of the DNA). The map below the histogram indicates the position of two nucleosomes on the two tandem repeats of the *Xenopus* 5S RNA gene. The arrows show the location and orientation of the 120 bp transcription unit for 5S RNA. The yellow ovals indicate the most frequent position of the nucleosome determined by the AFM analysis and the red circle indicates the site of biotin/streptavidin conjugation.

imal site is less mobile than that at the distal site [12]. Furthermore, the distal nucleosome was often found beyond the possible range, assuming that each nucleosome traps 146 bp DNA. This could be possible if the nucleosome contains a shorter DNA (~80 bp), enough for wrapping the core histone once (for detailed discussion, see Section 3.2).

The earlier work has demonstrated that an inclusion of linker histones into transcriptionally competent dinucleosomal templates represses the transcription. It is implicated that the linker histones restrict the nucleosome mobility and, thus, stabilize the histone-DNA contacts over the essential promoter elements [12,14]. An addition of histone H1 to the present reconstituted dinucleosome indeed resulted in further compaction with a medial spacing distance of 20 nm (Fig. 3C, center panel), consistent with the previous biochemical data [12]. In addition, using the same reconstituted dinucleosome as in this study, a selective repression of transcription from nucleosomal templates has been observed in the presence of

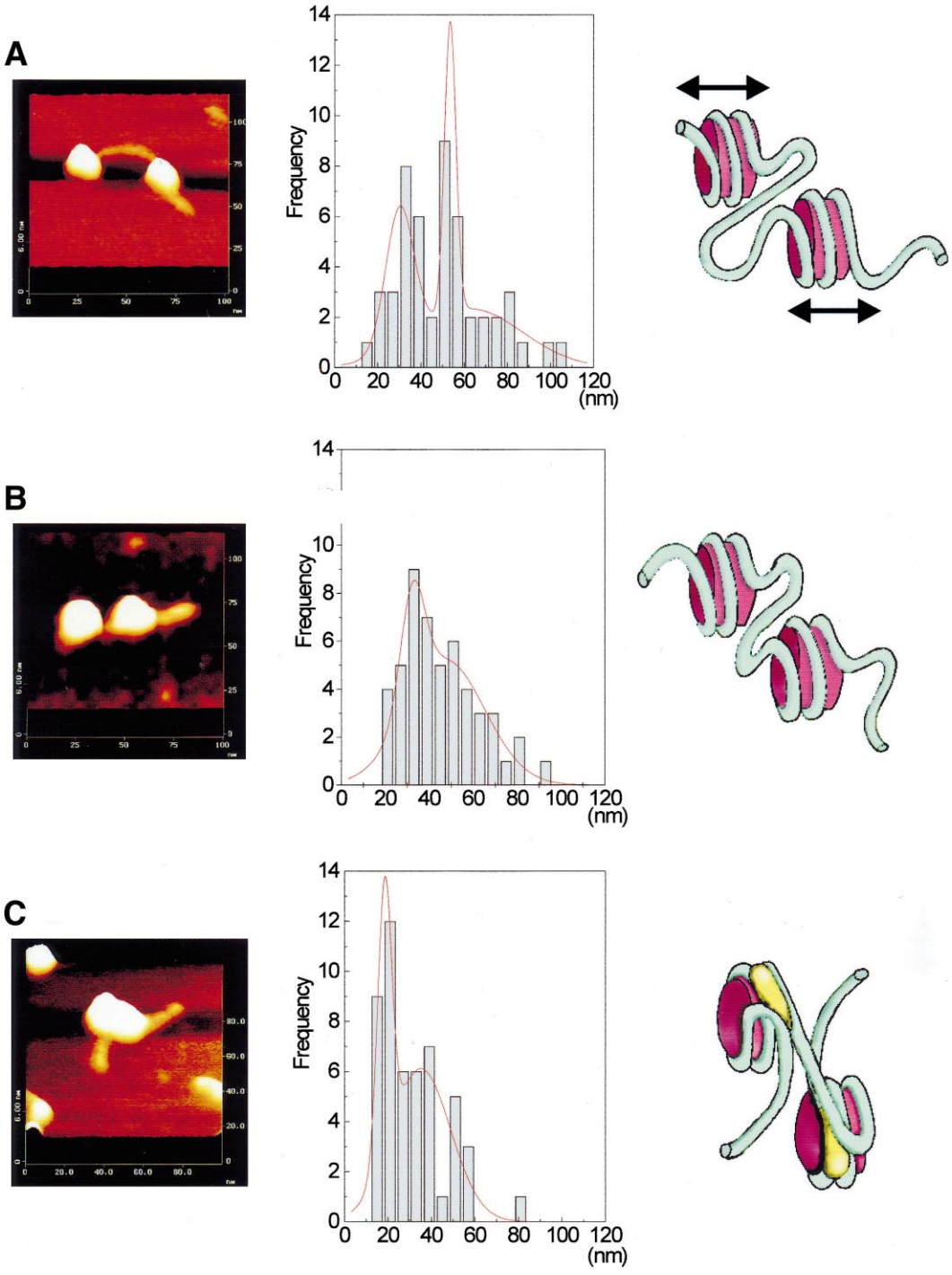

Fig. 3. An introduction of histone H1 to the reconstituted dinucleosomal system resulted in nucleosome compaction. Right panels: typical AFM images of dinucleosomes in 0 mM NaCl (A), 50 mM NaCl (B) or 50 mM NaCl plus linker histone H1 (C). Histone H1 was prepared from chicken erythrocytes by means of 5% perchloric acid extraction of nuclei and acetone precipitation [23]. The reconstituted nucleosome cores (1 μg of DNA) were incubated with 0.2 μg of histone H1 in 20 μl of binding buffer containing 10 mM Tris-Cl (pH 8.0), 50 mM NaCl, 0.1 mM EDTA at 26°C for 20 min. Center panels: statistical analysis of the internucleosomal length of dinucleosomes in 0 mM NaCl (A), 50 mM NaCl (B) or 50 mM NaCl plus histone H1 (C). The internucleosomal length was plotted against the number of measurements. The number of measurements (frequency) was given in 6 nm bins and the curves were obtained from a Gaussian fit. The peak positions are 34.5 and 53.4 nm in the absence of salt, 33.0 and 52.2 nm in the presence of 50 mM NaCl and 19.8, 36.6 and 53.6 nm with histone H1. It should be noted that an addition of histone H1 to the reconstituted system resulted in further compaction with a medial distance of 20 nm. Left panels: model for chromatin compaction induced by salt and linker histone H1. (A) The nucleosomes fluctuate along the template DNA in the absence of salt (see arrows). (B) The nucleosome is stabilized and located around the positional signals in 50 mM NaCl. (C) The linker histone incorporated into the nucleosome core interacts with the linker DNA via its amino- and carboxy-terminal tails and brings the two DNA duplexes (entering and exiting strings) together at the dyad region of the nucleosome.

linker histones [9,12]. The molecular AFM image shows that the two nucleosomes were closely packed to eachother by the addition of histone H1 and the linker DNA was not visible due to the mass of the nucleosomes. It is interesting to note that the DNA tail was often protruded from between the two attached nucleosomes (Fig. 3C, left panel). Since linker histone added to the nucleosome core is thought to interact with the linker DNA via its amino- and carboxy-terminal tails and brings the two DNA duplexes (entering and exiting strings) together at the dyad region of the nucleosome [18–22], we interpret that our AFM image is indeed indicating such a structure induced by histone H1 (Fig. 3C, right panel).

3.2. Quantitative analysis of nucleosome fluctuation and stabilization

A statistical analysis on the internucleosome distance provides further quantitative information for considering the organization of a dinucleosome. The frequency histogram of the distance between two nucleosome centers (the internucleosome distance) revealed the existence of two distinct spacings at a very low ionic strength (10 mM Tris-Cl, 0.1 mM EDTA). The two most frequent internucleosome distances were about 34 nm (type A) and 50 nm (type B) (Fig. 3A, center panel). The type A, but not type B, distance coincided with the length measured by the biochemical method [12]. In the presence of salt (50 mM NaCl), the population of dinucleosomes with a type B distance was reduced and that with type A spacing became the major form, indicating that most nucleosomes were localized around the two positioning signals (Fig. 3A, B, center panel).

Type B could be resulted from unfolded nucleosomes in the very low ionic condition [23]. The length of DNA wrapped around the core histone is shorter than 146 bp, probably, the DNA was wrapped once around the core histone. This was confirmed by analyzing a mononucleosome formed on the same template DNA. On the basis of the initial length of template DNA (148.2 ± 5.3 (S.D.) nm for 437 bp), the medial length of the mononucleosome at the distal end was calculated to be 123.6 nm in the low ionic condition, leaving 24.6 nm (∼72 bp) for one nucleosome formation.

In the presence of histone H1, the major population of the internucleosome length of the dinucleosome was about 20 nm (Fig. 3C, center panel). In this selected group of nucleosomes, the length of the linker plus tail DNA was 40.2 ± 19.0 nm (mean ± S.D. of 40 measurements), leaving the DNA (108 nm) corresponding to at least 320 bp for two nucleosome formations. Thus, the nucleosomes trapped more DNA in the presence than in the absence of histone H1.

Thus, we now established an AFM imaging procedure that allows us to not only visualize the chromatin structure but also quantitatively analyze nucleosome unfolding and compaction induced by very low salt and linker histone H1, respectively. This successful application of AFM to the structural analysis of reconstituted nucleosomes paved the way to exploring the structure-function relationships of specific genes at the chromatin level in vitro.

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