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Nucleotide and calcium-induced conformational changes in histone H1

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Abstract The principal constituents of chromatin, histone H1 (H1) and the nucleosome have essential roles in regulation of eukaryotic gene expression. However, mechanisms for the H1-dependent inactivation and for the ATP-dependent chromatin remodeling upon activation are largely unelucidated. Using circular dichroism (CD) analysis we show that ATP and other nucleotides and Ca^{2+} induce structural changes in H1. ATP and Ca^{2+} also induce changes when H1 is interacting with DNA, and the changes in H1 are accompanied by alterations in its DNA interaction. These results suggest that nucleotide and Ca^{2+} binding may be important for H1-mediated chromatin changes.

Key words: Nucleotide; Histone H1; Chromatin; Calcium; ATP; Circular dichroism

1. Introduction

The binding of histone H1 (H1) to nucleosomal DNA contributes to chromatin condensation into inactive solenoid-like structures [1,2]. H1 is also present in active chromatin, but the mode of interaction with nucleosomal DNA is presumably different [3]. The exact mechanisms by which the binding of H1 changes upon selective tissue-specific gene activation and chromatin decondensation are not established. Recent studies demonstrate that hydrolysis of ATP facilitates the nucleosome disruption and the binding of transcription factors in various reconstructed chromatin systems [4–7], suggesting that ATP is involved in chromatin remodeling. The ATP effect on nucleosome mobility and chromatin accessibility, likely due to conformational changes in histone proteins, also occurs in the presence of H1 [7]. However, the exact site(s) of ATP interaction upon activation of chromatin has remained unelucidated.

The approximately 220-amino-acid (aa) H1 proteins consist of a structured globular domain (gH1) and mobile and less structured N- and C-terminal extensions of approximately 30-aa and 100-aa, respectively [8]. The three-dimensional structures of chicken gH1 and gH5 (the globular domain of an avian reticulocyte-specific variant of H1) have been determined at high resolution using NMR spectroscopy and X-ray diffraction [9,10]. They contain three α-helices, and on the C-terminal side they contain a β-hairpin. The α-helical portion of gH5 and the DNA-binding motif of the prokaryotic cAMP receptor protein, CRP, can be superimposed [10], and gH1 is folded in a very similar manner [9], suggesting that the folding of gH1 and gH5 is important in their binding to DNA. Although the structures of gH1 and gH5 resemble the DNA-binding motif of CRP, the data suggesting an ability of H1 to bind to DNA in a sequence-specific manner is controversial [11,12]. The β-hairpin portion of gH1 resembles the phosphate-binding motifs of β-actin [13] and the catalytic subunit of cAMP-dependent protein kinase [14] involved in the recognition of ATP. There is also a limited amount of data supporting specific binding of nucleotides to H1 [15–17].

In the present report we have used circular dichroism (CD) spectroscopy to analyze if ATP and other nucleotides affect the structure of H1. We find that they can induce a high level of α-helicity in H1 and that ATP specifically affects the H1 structure also in the presence of DNA and/or Ca^{2+}. We also show that, in the presence of ATP or Ca^{2+}-ATP, H1 induces distinct changes in the CD spectrum of DNA, indicative of changes in the DNA structure.

2. Materials and methods

Perchloric acid-soluble proteins containing mainly H1 were extracted from rat liver nuclei as described [18], and H1 was separated from high-mobility group proteins by electrophoresis on a reverse-phase column using a Kontron HPLC apparatus [19]. Nucleotides and analogues were purchased from Sigma Chemical Co. (St. Louis, MO) or Boehringer-Mannheim GmbH (Mannheim, Germany). Poly-(dA-dT) and poly(dI-dC) were purchased from Pharmacia (Uppsala, Sweden).

CD spectra in the wavelength ranges of 183-260 or 183-300 nm were recorded on a JASCO J-600 spectropolarimeter. The band width was 1.0 nm, and the step resolution was 0.5 nm. Quartz cuvettes with an optical path length of 0.1 cm were kept at room temperature. The histone H1 concentration was 200 μg/ml, and the buffer contained 20 mM NaCl and 5 mM Tris-HCl, pH 7.5, plus the specified additions. EDTA (0.1 mM) was present when Ca^{2+} or Mg^{2-} was not added. All spectra were corrected with the instrumental base line. The background spectrum of all added reagents were recorded at the concentrations used and substracted from the corresponding spectrum with H1 present in order to calculate the difference spectrum. From difference spectra the degree of secondary structure was calculated by using the commercial software from JASCO, version 302, which is based on the methods given in references [20] and [21].

3. Result and discussion

3.1. Non-physiological concentrations of inorganic phosphate (P_i) increase the α-helicity of H1

Very high concentrations of inorganic phosphate (P_i) were used in the determinations of the gH1 and gH5 structures by NMR and X-ray crystallography [9,10]. It has also been shown that P_i can affect the secondary structure of H1 and H5 [9,10,15]. Therefore, we used circular dichroism analysis to investigate the concentration dependence of the P_i effect on the structure of rat liver H1 and if it could be obtained also at physiological P_i concentrations. The presence of 10 mM sodium phosphate (P_i) had a large effect on its CD spectrum (Fig. 1A). The changes obtained in the presence of P_i are characteristic of a large increase in the α-helicity of H1. We calculated its α-helicity from the difference spectrum of H1 at

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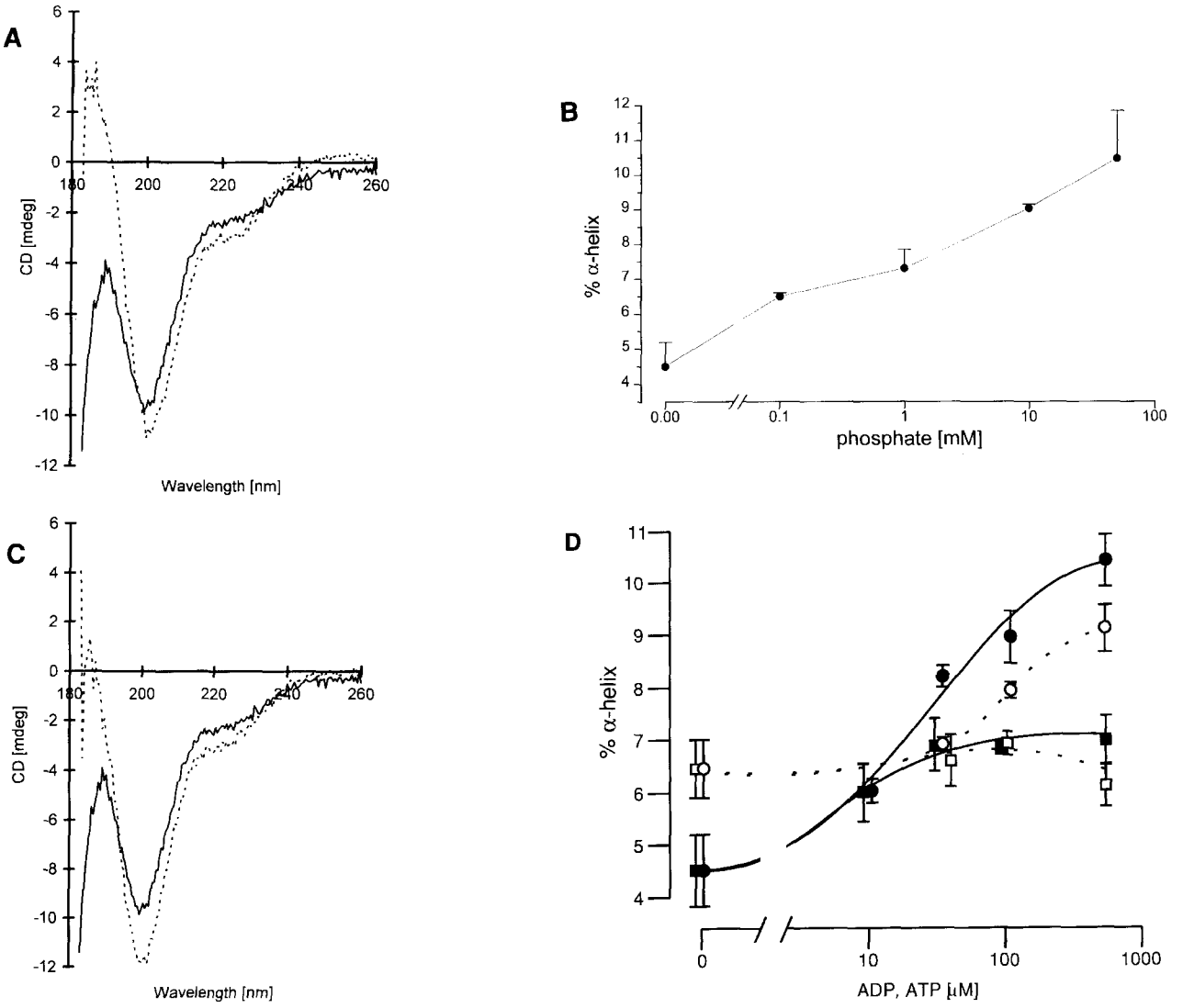

Fig. 1. Effects of P_i, ATP, ADP and Ca^{2+} on the secondary structure of H1. A: CD spectra of H1 in the absence of P_i (continuous line) and in the presence of 10 mM sodium phosphate (P_i) (discontinuous line; CD spectrum of 10 mM P_i subtracted). The CD spectra are averages of three determinations. B: The percentage of α-helix in H1 in the presence of increasing concentrations of P_i. C: CD spectra of H1 in the absence of ATP (continuous line) and in the presence of 100 μM ATP (discontinuous line; CD spectrum of 100 μM ATP subtracted). D: Effects of increasing concentrations of ATP (circles) and ADP (squares) on the α-helicity of H1 in the absence (closed symbols and continuous lines) and presence of 50 μM $CaCl_2$ (open symbols and discontinuous lines). In (B) and (D) the percentages of α-helix were estimated from at least three CD determinations, with subtraction of the CD spectra of the added concentrations of P_i, ATP, ADP, and $CaCl_2$. The bars indicate the standard deviations.

various P_i concentrations [20,21]. The α-helicity increased gradually over a broad range of P_i concentrations. Without P_i in the solution, only 4.5 ± 0.7% of the protein appeared folded into α-helix and an increase up to 10.5% was observed when the P_i concentration was increased to 50 mM (Fig. 1B). These values correspond to approximately 10-aa and 23-aa residues, respectively, in an α-helical form. Thus, the P_i concentration required for folding of the α-helices of the DNA-binding motif in gH1 is much above the physiological intracellular concentrations of P_i. Therefore it seems unlikely that P_i promotes the folding of these α-helices in vivo.

3.2. Effect of nucleotides on the conformation of H1

As the nucleoside triphosphate ATP is present at mM concentrations in the nucleus, we investigated its ability to promote the folding of H1 at physiological or sub-physiological concentrations. As little as 100 μM ATP produced corresponding effects on the CD spectrum of H1 as 10 mM sodium phosphate (P_i) did (cf. Fig. 1A,C). An increase in the α-helicity of H1 to 10.4 ± 0.5% was obtained at a concentration of 500 μM ATP (Fig. 1D). Thus, the increase in α-helicity could be produced with 100-fold lower concentrations of ATP than P_i. ATP alone showed very small circular dichroism at concentrations where it had dramatic effects on the difference spectrum of H1 (data not shown).

To determine the specificity of the ATP-induced increase in the α-helicity of H1, we analyzed the effects of various related substances. ADP did also increase the α-helicity, although the effect was smaller than for ATP (Fig. 1D and 2). A maximal α-helicity increase of less than 3% could be detected. The effect of ADP reached a plateau already at a concentration of 33 μM (Fig. 1D). A level of the α-helicity effect similar to

that of ADP was also obtained with AMP or ribose-5-phosphate, but the effect was smaller with adenosine, ribose or adenine (Fig. 2A and data not shown). An effect close to that with ATP was also seen with dATP and GTP, whereas ddATP showed an intermediate effect (Fig. 2A). These results indicate that ribose and the α-phosphate are responsible for the effect of ADP. Comparison of the results with ATP and ADP show that the γ-phosphate of ATP is required to obtain the full structural changes in H1. The nucleotide base was insignificant in the binding of nucleotide to H1.

3.3. DNA promoted folding of H1

Various different models have been proposed concerning the binding of H1 or H5 to nucleosomal DNA. H1 is believed to bind preferentially to AT-rich regions of DNA, at least partly through multiple SPKK motifs in its N- and C-terminal extensions [22,23].

Addition of double-stranded poly(dA-dT) or poly(dI-dC) DNA to H1 resulted in an increase in the α-helicity of H1 (Fig. 2B and 3A). It increased from 4.5% up to a maximum of 9.4 ± 0.2% and 8.3 ± 0.6% respectively. Increasing DNA concentrations increased linearly the α-helicity of H1 up to a concentration where the H1-binding sites on the DNA could be estimated to correspond to the amount of H1 (data not shown). The similar effects with poly(dA-dT), the preferred DNA sequence for H1 [24], and the non-specific DNA, poly- (dI-dC), suggests that the ability of DNA to promote the folding of H1 is not dependent on a specific poly(dA-dT) DNA interaction.

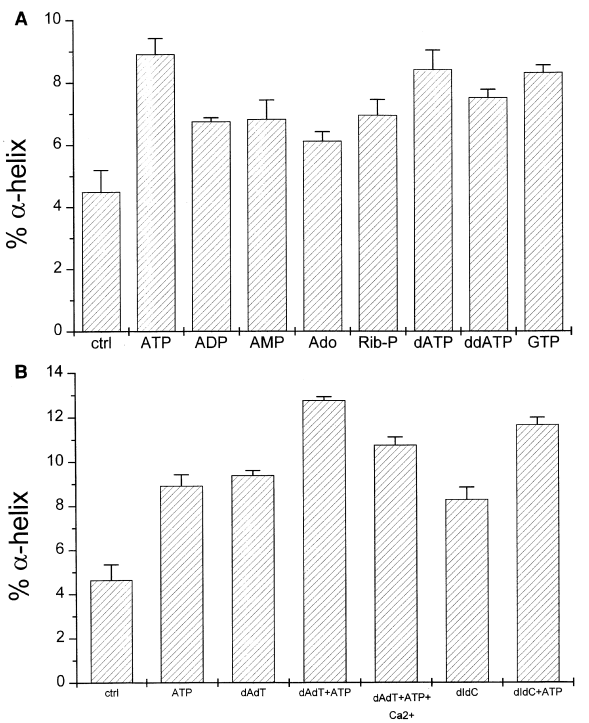

Fig. 2. A: The percentage of α-helix in H1 in the presence of a 100 μM concentration of various nucleotides and their derivatives. Ado, adenosine; Rib-P, ribose-5'phosphate. B: Effects of 100 μM ATP and 50 μM $CaCl_2$ on the α-helicity of H1 in the presence of poly-(dA-dT) or poly(dI-dC). Averages and standard deviations of at least three determinations, with subtraction of the CD spectra of the added concentrations of nucleotides and their derivatives, $CaCl_2$, poly(dA-dT) and/or poly(dI-dC), are indicated.

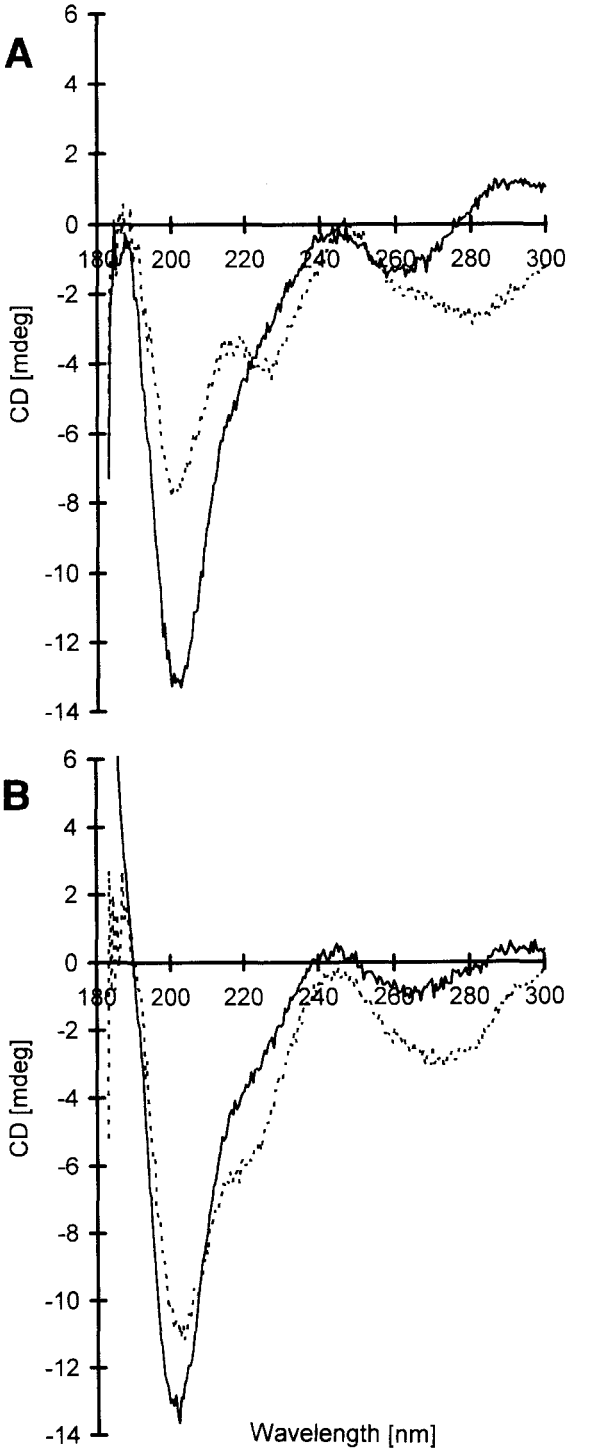

Fig. 3. Effects of ATP and Ca^{2+} on the CD spectra of H1 in the presence of poly)dA-dT). A: CD spectra of H1 in the presence of poly(dA-dT) at a 1:1 ratio in the absence of ATP (continuous line; CD spectrum of poly(dA-dT) subtracted) and in the presence of 100 μM ATP (discontinuous line; CD spectrum of poly(dA-dT) plus 100 μM ATP subtracted). B: The same as (A) in the presence of 50 μM $CaCl_2$.

3.4. Specific effect of ATP on H1

From the CD analyses presented it is evident that each of P_i, ATP and DNA can induce changes in the H1 structure. We therefore investigated if the ATP-induced conformational change in H1 is specific for the nucleotide, or if alternatively

P_i or DNA can replace the ATP. Addition of 100 μM ATP increased the α-helicity of H1 from 7.3 ± 0.6% to 9.9 ± 0.2% in the presence of 1 mM P_i and from 9.0 ± 0.1% to 10.8 ± 0.4% in the presence of 10 mM P_i. Thus, the effect of ATP is distinct from the effect of P_i. The effect of ATP was also distinct from the effect of DNA, since an increase of approximately 4% in the α-helicity of H1 was observed upon addition of 100 μM ATP in the presence of double-stranded poly(dA-dT) or poly(dI-dC) DNA (Fig. 2B and 3). Furthermore, ATP induced a slight increase in the α-helicity of H1 in the simultaneous presence of P_i and DNA (data not shown).

3.5. Ca^{2+}-ATP-induced conformational changes in H1

A divalent cation, Mg^{2+} or Ca^{2+}, is at least in most cases involved in the binding of ATP to proteins with ATP-dependent activity, such as protein kinases and actin [25]. In a previous study Mg^{2+} had little effect when the ability to stabilize the binding of nucleoside triphosphate to H1 was investigated [26]. In accordance with those results, Mg^{2+} did not produce any change in the folding of H1 in our CD analysis even at a concentration of 1 mM (data not shown). However, in contrast to Mg^{2+}, Ca^{2+} changed the conformation of H1. 50 μM $CaCl_2$ in the presence of a high excess of $NaCl_2$ increased the α-helicity of H1 from 4.5 to 6.5% in the absence of nucleotide (Fig. 1D). ATP promoted an increase in the α-helicity of H1 also in the presence of 50 μM $CaCl_2$, and $CaCl_2$ decreased the α-helicity of H1 also in the presence of ATP plus poly(dA-dT) (Fig. 1D and 2B). The effect of ATP was slightly lower in the presence of $CaCl_2$ than without it (Fig. 1D, 2B and 3). We do not at present have an explanation for the lower effect of ATP in the presence of Ca^{2+}, but it is likely to be coupled to the changes in the tertiary structure of gH1. However, Ca^{2+} together with ATP may also change the conformation of H1 in vivo.

3.6. H1-induced structural changes on DNA in the presence of ATP, Ca^{2+} and Ca^{2+}-ATP

The CD analyses also allowed estimation of the effects of H1 on DNA conformation, by monitoring changes between 260 and 300 nm, where signals arise almost exclusively from the DNA bases [27]. Addition of H1 resulted only in a minor deflection in this particular region of the CD spectrum of double-stranded poly(dA-dT), whereas no change was obtained in the CD spectrum of poly(dI-dC) (data not shown). However, addition of 100 μM ATP to H1 plus poly(dA-dT) induced a large reduction of positive ellipticity in the calculated difference spectrum of H1, with the CD spectrum of poly(dA-dT) and poly(dA-dT) plus ATP subtracted, respectively, exhibiting a negative maximum at ~280 nm (Fig. 3A). The drastic decrease in ellipticity induced by ATP suggests a significant conformational change in the DNA. This is possibly coupled to the large conformational change in H1 obtained in the same CD spectrum and due to alterations in the interaction of H1 with DNA. Furthermore, as the ATP-mediated folding of H1 changes the structure of DNA, it strongly argues against the possibility that the effect of ATP in H1 is derived from nonspecific ATP binding to the DNA-binding regions of H1. When 50 μM $CaCl_2$ was added to H1 plus poly(dA-dT), the positive and negative ellipticities of the calculated difference spectrum of H1 were a little smaller than without Ca^{2+} but there was no considerable shift of the peaks (Fig. 3B). The difference spectrum of H1 in the presence of poly(dA-dT), Ca^{2+} and ATP was similar to the spectrum in the presence of poly(dA-dT) and ATP, although there was a little shift to the shorter wavelengths with the negative maximum being at ~275 nm (Fig. 3B). When ATP, Ca^{2+} or Ca^{2+}-ATP was added to H1 in the presence of poly(dI-dC), no larger change than the experimental error of the assay could be detected in the CD spectrum (data not shown). This observation is in accordance with the data that H1 preferentially binds through its N- and C-terminal extensions to poly(dA-dT) [22,23]. The ability of H1 to change the structure of the poly(dA-dT) could indicate that H1 may bind to it through more than one contact point.

3.7. Functional implications of H1

It is interesting, that the most abundant nucleotide in the nucleus, ATP, promotes the folding of H1 in vitro at concentrations even below its in vivo concentration, and that it can also change the structure of H1–DNA complexes. Since formation of the reported crystal and solution structures of gH1 and gH5 [9,10] are based on the use of higher than physiological P_i concentrations, it is more likely that ATP is promoting folding of the globular domain of H1 in vivo. Although the folding of gH1 and gH5 might not necessarily occur in vivo to the extent previously presented [9,10], it is obvious that the folding of gH1 is important in the H1–DNA interaction.

The conformational change of H1 appears to alter the H1–DNA interaction and to distort poly(dA-dT) DNA. This observation is interesting, as poly(dA-dT) tracts are conformationally rigid and they are preferentially positioned at the ends of the nucleosome core and at the linker DNA [28]. Our data is in accordance with the observations [29,30] that in active chromatin H1 interacts through its tails with AT-rich DNA regions while its interaction with the central globular domain is altered. Moreover, it should be pointed out that segments of poly(dA-dT) occur quite frequently in regulatory regions of genes. Thus, the present results are in accordance with the observations that ATP is involved in chromatin remodeling upon activation [4–7]. Since the effect of ATP has been coupled directly to nucleosome movement in the presence of H1 [7], it is evident that H1 could be one of the ATP-binding entities or could form part of an ATP-binding site [13].

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