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Rat liver HMG1: a physiological nucleosome assembly factor

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Incubation of rat liver single-stranded DNA-binding protein HMG1 with the four core histones at 0.15 M NaCl favors histone association primarily into tetramers and, to a lesser extent, into octamers. The assembly of pre-formed histone-HMG1 complexes with DNA yields nucleosome-like subunits which satisfy most of the criteria defining native core particles: (i) the circular DNA extracted from the complexes is supercoiled indicating that the initially relaxed DNA acquired superhelical turns during complex formation in the presence of topoisomerase I; (ii) the digestion of the complexes with micrococcal nuclease yields a DNA fragment of ~140 bp in length; (iii) electron microscopy of the reconstituted complexes shows a beaded structure with the DNA wrapped around the histone cores, leading to a reduction in the contour length of the genome compared with free DNA. Moreover, in the presence of HMG1, nucleosome assembly occurs rapidly at 0.15 M NaCl. Therefore, in addition to its DNA-binding properties, HMG1 mediates the assembly of nucleosomes *in vitro* under conditions of physiological ionic strength. The possible involvement of these properties in the DNA replication process is discussed.
Key words: chromatin replication/HMG1 protein/histone-HMG1/DNA interactions/nucleosome assembly

Introduction

The basic structure of eucaryotic chromatin is now reasonably well understood at the nucleosomal level (for a review, see McGhee and Felsenfeld, 1980). However, the dynamic aspects of nucleosome assembly and the rearrangements that occur during biological processes are poorly understood. The process of chromatin assembly *in vitro* from the separated components has been widely studied. Direct mixing of DNA with histones leads to non-specific aggregation and precipitation of protein-DNA complexes at low ionic strength. Consequently, the current approach to reconstituting nucleosome core particles consists of a lengthy dialysis of DNA and histones from high salt, in the presence or absence of urea. These conditions allow stabilization of histone octamers by neutralizing the strong electrostatic interactions between DNA and histones (Oudet *et al.*, 1975; Germond *et al.*, 1975; Camerini-Otero *et al.*, 1976). On the other hand, Ruiz-Curillo *et al.* (1979) demonstrated that nucleosome assembly occurred at physiological ionic strength if the rate of mixing of DNA and histones was sufficiently low to prevent precipitation and allow their correct association.

Assembly of newly replicated DNA into nucleosomes *in vivo* is presumably a relatively rapid process. Rapid assembly of nucleosomes *in vitro* at physiological ionic strength is facilitated by several different factors. The first of these is nucleoplasmin, an acidic thermostable protein, which is the predominant nuclear protein of *Xenopus* oocytes (Laskey *et al.*, 1978). It interacts with histones *in vitro* and appears to act by preventing non-specific aggregate formation between DNA and histones, thus facilitating an ordered assembly process. Nucleoplasmin binds neither to DNA nor to chromatin. RNA also mediates chromatin assembly (Nelson *et al.*, 1981), a property which was first ascribed to DNA topoisomerase I (Germond *et al.*, 1979). Acidic polypeptides also stabilize histone octamers at physiological ionic strength and facilitate the assembly of core particles (Stein *et al.*, 1979). In keeping with this last observation, we tested the ability of the chromatin protein HMG1 to facilitate the assembly of nucleosomes *in vitro*.

Knowledge of the amino acid sequence of HMG1 has revealed a striking asymmetry in the distribution of amino acids (Walker *et al.*, 1980). The presence of structural domains in HMG1 has been proposed, namely N-terminal and central domains which contain the majority of the hydrophobic and basic residues in the protein and may be responsible for the binding to DNA, and a highly acidic C-terminal domain containing a sequence of 41 consecutive acidic residues which may be involved in complex formation with the very basic regions of histones (Reeck *et al.*, 1982; Cary *et al.*, 1983; Carballo *et al.*, 1983). In fact, interactions between HMG1 and histone H1 have been reported (Shooter *et al.*, 1974; Smerdon and Isenberg, 1976), but, HMG1 was not found to be active in nucleosome assembly *in vitro* (Nelson *et al.*, 1981). The methods used for the purification of this protein could be responsible for its inactivity [exposure to extremes of pH and organic solvents (Goodwin *et al.*, 1973)].

We have previously described the purification and properties of a single-stranded DNA-binding protein (SSB) from rat liver, isolated under conditions which preserved native structure (i.e., differential DNA cellulose affinity chromatography and phosphocellulose chromatography) (Duguet and De Recondo, 1978). This allowed us to show that the different functional properties exhibited by this protein depended upon the physiological state of the rat liver. The SSB protein isolated from regenerating rat liver (called HD25) lowered the melting point of poly [d(A-T)] and stimulated rat liver DNA polymerases α and β *in vitro* (Duguet *et al.*, 1977). The SSB protein extracted from normal rat liver (called S25), did not lower the *T*m of poly [d(A-T)] and inhibited DNA polymerases (Bonne *et al.*, 1979; De Recondo *et al.*, 1980). HD25 and S26, indistinguishable in most respects, were recently identified as the rat liver HMG1 protein (Bonne *et al.*, 1982).

The present work provides evidence that HMG1 also interacts with histones and is able to mediate the reconstitution of subunits very similar to nucleosome cores at physiological ionic strength.

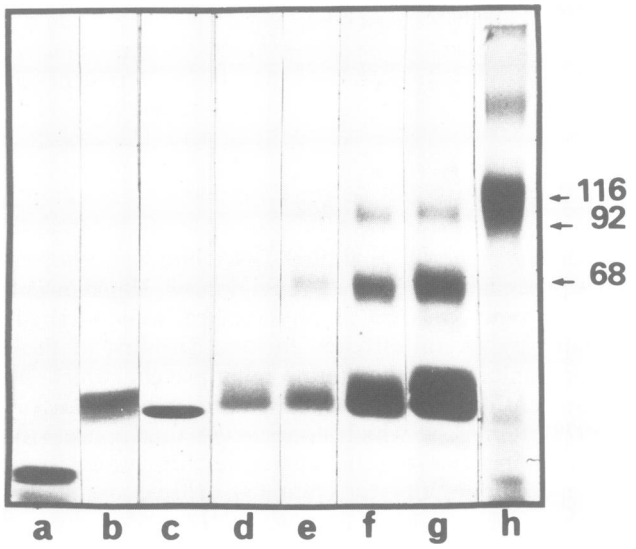

Fig. 1. Effect of HMG1 protein on the cross-linking of histones at 0.15 M NaCl. Concentrated histones in a 10 mM Tris-HCl, pH 8, 2 M NaCl buffer, were diluted in a HMG1 solution to 0.15 M NaCl, pH 7.5, at different appropriate weight ratios. Samples were cross-linked with dimethyl suberimidate, as indicated in Materials and mthods, prior to addition of SDS and analysis on an SDS 4–20% polyacrylamide gradient gel. **Lanes a** and **b**, histones (10 μg) untreated and treated with dimethyl suberimidate; **lane c** and **d**, HMG1 (5 μg) untreated or treated with dimethyl-suberimidate; **lanes e, f** and **g**, histones (10 μg) mixed respectively with 5, 10 and 20 μg of HMG1 and treated with dimethyl-suberimidate; **lane h**, cross-linked histone octamer marker, obtained from core particles purified as described in Materials and methods, and treated with dimethyl suberimidate. Marker proteins electrophoresed in adjacent lanes and indicated by their mol. wts. (x 10^{-3}), were: *E. coli* β galactosidase, phosphorylase a and bovine serum albumin.

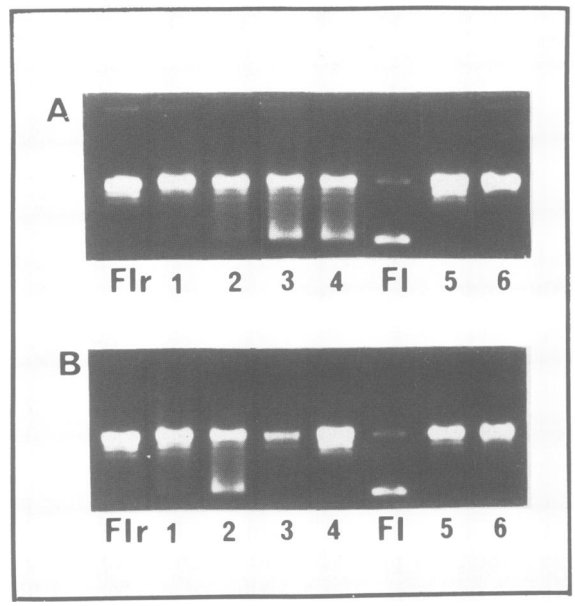

Fig. 2. Generation of superhelical turns in SV40 DNA Ir, due to core particle assembly at 0.15 M NaCl. Incubations were performed at 37°C for 30 min. SV40 DNA Ir was at a final concentration of 10 μg/ml and was added with topoisomerase I to pre-incubated histone-HMG1 solution. After incubation, DNA was deproteinized and electrophoresed as described in Materials and methods. **(A)** Supercoiling induced with increasing amounts of HMG1 at a prefixed histone-to-DNA weight ratio of 1.5. **Lanes 1–4**, final HMG1-to-histone weight ratios were 0.5 **(1)**, 1.0 **(2)**, 2.0 **(3)**, 3.0 **(4)**; **lane 5**, histones were omitted; **lane 6**, HMG1 was omitted. **(B)** Supercoiling induced at a prefixed HMG1-to-histone weight ratio of 2 with increasing amounts of histones. **Lanes 1–4**, final histone-to-DNA weight ratio of 0.8 **(1)**, 1.5 **(2)**, 2.0 **(3)**, 3.0 **(4)**, **lane 5**, histones were omitted; **lane 6**, HMG1 was omitted. The markers were supercoiled DNA (FI) and circular, relaxed, covalently closed DNA (FIr).

Results

Histone-histone associations at 0.15 M NaCl in the presence of HMG1 protein

The cross-linking reagent dimethyl suberimidate was used to reveal interactions among the histones in the presence or absence of HMG1. Optimal conditions for histone cross-linking at 0.15 M NaCl were determined using native chromatin, and HMG1-to-histone ratios were chosen with reference to those determined for the nucleoplasmin (Earnshaw *et al.*, 1980) or with acidic polypeptides (Stein *et al.*, 1979).

Figure 1 shows the results of such experiments, analyzed by SDS-PAGE: at 0.15 M NaCl, histones were largely cross-linked into dimers, whereas HMG1, under the same conditions, appeared largely at the monomer position. An extremely slight band could be seen at the position of an HMG1 trimer, as recently described by Bernues *et al.* (1983); this band remained barely detectable even in overloaded gels. When histones and HMG1 were mixed at HMG1-to-histone ratios of 0.5, 1 and 2, two major additional cross-linked products were revealed, which correspond to tetramers and octamers of histones on the basis of their respective electrophoretic mobilities. This result shows that HMG1 favors the association of histones at low ionic strength.

The relative yield of the tetramer and octamer appeared to be primarily proportional to the amount of histones, and was independent of the time and temperature of incubation. As suggested by the relative proportion of HMG1 at the monomer position (samples e, f and g), the protein did not seem to be cross-linked with histones. In addition, HMG1 was not detected in the cross-linked histone tetramer and octamer, by immunochemical techniques (not shown), but this result could be explained by the inaccessibility of HMG1 antigenic determinants in the complexes.

As shown in lane h, histones in native core particles were cross-linked, only into an octamer form showing that the cross-linking procedure has been completed.

Assembly of nucleosome

We next questioned whether HMG1 was able to mediate the transfer of associated histones to DNA and to reconstitute nucleosome subunits at physiological ionic strength.

Supercoiling assay. Nucleosome formation in the presence of topoisomerase I was followed by the insertion of superhelical turns in circular DNA extracted from reconstituted complexes as described by Germond *et al.* (1979).

Histones pre-incubated with HMG1 were mixed with SV40 relaxed covalently closed DNA (DNA Ir) at an ionic strength of 0.15 M NaCl. At this salt concentration, the ability of HMG1 alone to induce supercoiling of DNA was totally inhibited (Duguet *et al.*, 1981). Topoisomerase I was added to the mixture to relieve extranucleosomal superhelicity. Since our fractions of topoisomerase I were active in assembly (Germond *et al.*, 1979), we determined for each experiment the amount of topoisomerase I required to relax DNA without concomitant assembly of nucleosomes during a fixed incubation interval. The deproteinized DNA was then analyzed by agarose gel electrophoresis as described in Materials and methods.

Figure 2 shows the distribution of superhelical turns induc-

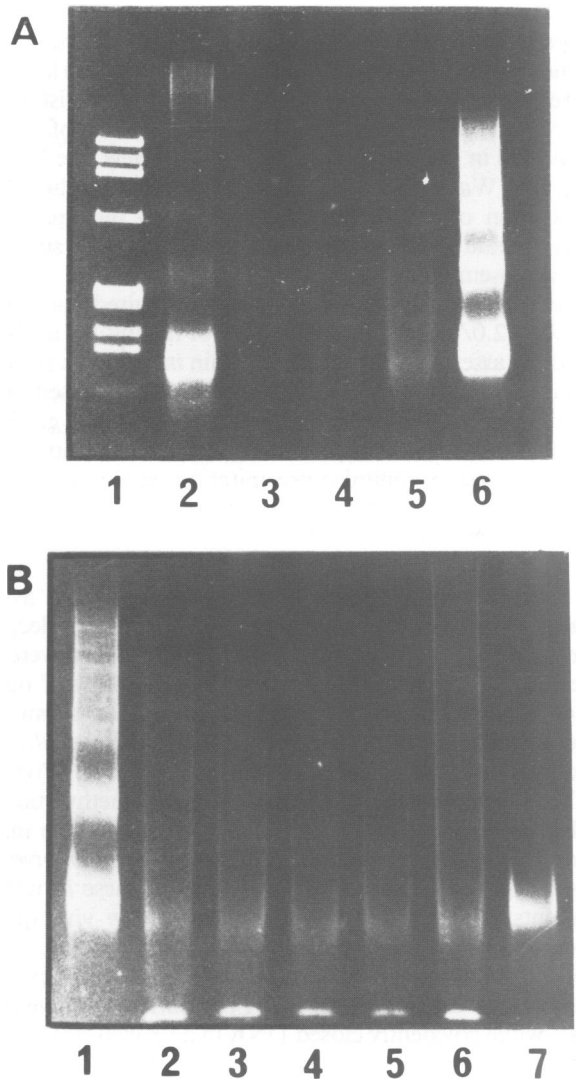

Fig. 3. (A) Nuclease digestion of the products of *in vitro* assembly. SV40 DNA (1 μg), either supercoiled or relaxed, was added to histone-HMG1 solution. Histone-to-DNA weight ratio was 1.2 and HMG1-to-histone weight ratio was 2.0. After an incubation at 37°C for 30 min, samples were digested with micrococcal nuclease and prepared for electrophoresis as described in Materials and methods. **Lane 1,** *Hae*III-digested φX174 DNA; **lane 2,** DNA isolated from native core particles (140–200 bp); **lane 3,** assembly with DNA Ir supplemented in topoisomerase I; **lane 4,** assembly with DNA Ir; **lane 5,** assembly with DNA I; **lane 6,** DNA isolated from micrococcal nuclease digestion of rat liver chromatin. **(B)** Effect of assembly time on the level of *in vitro* nucleosome reconstitution. Assembly was performed with ^{3}H-labelled SV40 DNA I under conditions described above during various times of incubation. At each time nuclease attack was performed under standard conditions described in Materials and methods; an aliquot was removed from a large-scale reaction: 0.1 μg for measuring the amount of DNA protected by acid precipitation and 0.9 μg for analyzing the DNA fragments on acrylamide gel. **Lane 1,** DNA fragments isolated from micrococcal nuclease digestion of rat liver chromatin. **Lanes 2–6,** the times of *in vitro* assembly prior to digestion were 0.5, 30, 60 and 90 min; **lane 7,** DNA of native core particles. Zero time is the time necessary to take an aliquot for immediate digestion after the addition of DNA to histone-HMG1 solution.

ed in SV40 DNA Ir molecules by incubation with a mixture of HMG1-histones and treatment with topoisomerase I. The most efficient assembly was obtained at a histone-to-DNA weight ratio of 1.5 (Figure 2B, lane 2), and a HMG1-to-histone weight ratio of 2–3 (Figure 2A, lanes 3–4). These different weight ratios were only estimates, due to the uncontrolled loss of histones resulting from adhesion to the tubes during incubation. Lanes 3–4 in Figure 2B show that assembly was inhibited at a histone-to-DNA ratio higher than 1.5, even when HMG1 was added simultaneously with histones. When HMG1 was omitted from the association mixture, no supercoiling was observed (lane 6). Moreover, no change was observed in the initial relaxed state of the DNA when it was incubated with HMG1 alone at the same salt concentration (lane 5, Figure 2A, B). Both fully supercoiled and relaxed species were present regardless of the ratio of protein to DNA used in these assays. The assembly process was never complete even by increasing the time of incubation (see Discussion).

Micrococcal nuclease digestion of reconstituted complexes. To confirm the fidelity of core particle reconstitution in the presence of HMG1 *in vitro*, we next determined the size of DNA fragments released from the reconstituted complex after micrococcal nuclease treatment.

Furthermore, to prove that the extract containing topoisomerase I was not required in the assembly process, complexes were reconstituted from a mixture of the four histones, HMG1, and SV40 DNA I, the latter being either relaxed (with or without topoisomerase) or supercoiled. The reconstituted protein-DNA complexes were digested with micrococcal nuclease under standard conditions as described in Materials and methods. As shown in Figure 3A, only the complexes reconstituted with supercoiled DNA yielded an assembly sufficient to permit observation of a protected DNA fragment in the monomer region (lane 5); with DNA Ir, with or without topoisomerase I, DNA fragments of 140 bp were barely detected under these experimental conditions (lanes 3,4). When HMG1 or histones were omitted, no protected DNA fragment appeared (not shown). The size of the protected fragment released from the reconstituted complexes was similar to that of the fragment released from native nucleosomes (lanes 2,6). To obtain a quantitative measure of the extent of assembly under the different conditons of Figure 3A, similar experiments were performed with ^{3}H-labelled DNA. The percentages of acid-precipitable DNA protected from micrococcal nuclease were 20%, 5–10% and 35–40% for nucleosome complexes reconstituted under the conditions corresponding to Figure 3A, lanes 3, 4 and 5 respectively. In the absence of histones and HMG1, only 0–2% of the DNA was nuclease resistant. Topoisomerase I thus appeared to enhance the assembly of nucleosomes *in vitro* with a relaxed DNA, but the extent of assembly obtained is lower than that obtained with a negatively supercoiled DNA. The protein-to-DNA weight ratios for optimal assembly with supercoiled DNA seemed to be approximately the same as those determined with relaxed DNA in the supercoiling assay; higher amounts of histones always led to DNA precipitation. Since SV40 DNA contains ~5000 bp, and 140 bp were protected in each core particle, the percentage (35–40%) of protected Form I DNA corresponded to an average of 10–15 nucleosomes per SV40 genome.

The conversion of labelled SV40 DNA into a structure which yields a micrococcal nuclease-resistant DNA fragment was used to determine the rate of nucleosome assembly *in vitro* as described by Nelson *et al.* (1981) and Earnshaw *et al.* (1980). The resulting products were analyzed on acrylamide gels, while a separate aliquot was acid-precipitated and counted. The percentage of protected DNA measured at the earliest time of assembly (<5 min) was 35–40%, and did

not change with increasing times up to 90 min. As shown in Figure 3B, the same amount of 140-bp DNA fragment was observed at each time, indicating that the assembly process occurred very rapidly when histones were reconstituted with supercoiled DNA in the presence of HMG1.

Electron microscopy of reconstituted complexes. To demonstrate further that histones and supercoiled DNA are rapidly assembled in the presence of HMG1, SV40 DNA I was added to a solution of histones and HMG1 and the reconstituted complexes were directly examined by electron microscopy.

The electron micrographs shown in Figure 4 revealed that the histone complexes formed on SV40 DNA I (panels B–G) are globular particles morphologically similar to the native nucleosome (panel A) and with the same diameter of 100 Å. The beads were randomly distributed along the molecules and appeared either as individual beads connected by segments of DNA of irregular length (panels F–g), or as clusters of highly packed beads with no bridging DNA (panels D,E).

The number of nucleosomes per SV40 DNA molecule varied from 0 to 22, but the majority of the nucleoprotein complexes contained 9–14 nucleosomes. This was consistent with the amount of DNA protected against micrococcal nuclease. Each nucleosome contained ~140 ± 10 bp, as determined from the length reduction of internucleosomal DNA as a function of the number of nucleosomes. This is also in agreement with the size of the DNA fragments obtained after micrococcal nuclease digestion.

Discussion

Our results show that in the presence of HMG1, histones are able to associate in tetramers and octamers at physiological ionic strength. The apparent absence of cross-linking between HMG1 and histones, after addition of dimethyl-suberimidate may be due to the lack of lysine residues susceptible to amidination by the reagent in the region of HMG1 which interacts with histones. This is in agreement with the hypothesis that histones could bind to numerous aspartic and glutamic residues of the C-terminal region of HMG1. As expected, we found that the native conformation of HMG1 was essential for allowing assembly of the histones at low ionic strength: when HMG1 was denatured by trichloroacetic acid (TCA), it was completely inactive. As proposed for the other assembly factors, it seems probable that ionic interactions between HMG1 and histones are preponderant and that HMG1 binds histones in such a way that it neutralizes their basic charges, allowing their association at physiological ionic strength. According to this hypothesis, the amount of consecutive negative charges on the molecule would be the major factor determining the ability of the protein to act as an assembly factor. In the cross-linking experiments, HMG1 appeared to exist as a monomer: thus, it is not surprising that it neutralized only the charge of one histone tetramer by its acidic C-terminal region. To determine the stoichiometry of binding and, consequently, the charge distribution within the complexes, a more detailed study of histone binding to HMG1 is presently being pursued.

Although HMG1 seems to assemble the major part of the histones into tetramers, the DNA was rapidly folded into structures quite similar to native core particles when added to HMG1-histone complexes. This suggests that the transfer to DNA does not require the formation of a complete octamer, which would be promoted by the presence of DNA. The small quantity of octamer revealed by cross-linking could not account for the amount of nucleosome assembly observed. The *in vivo* study of Worcel *et al.* (1978) gives evidence that histones H3 and H4 are assembled at replication forks slightly before histones H2A and H2B. This result is consistent with the predominant role attributed to the tetramer of histones H3 and H4 in nucleosome structure (for review, see De Pamphilis and Wassarman, 1980). A knowledge of the histone composition of the products associated in the presence of HMG1 would provide arguments consistent with such a sequential assembly.

The HMG1-to-histone weight ratio required for optimal assembly (2.0/1) is of the same order of magnitude as that required for assembly with nucleoplasmin *in vitro*. As shown by the supercoiling assay, electron microscopy, and sedimentation of the reconstituted complexes on sucrose gradients, assembly was never complete. In fact, the conditions determined *in vitro* to minimize precipitation were restrictive and imposed a low final DNA concentration (< 10 μg/ml) and an average weight ratio of histone to DNA below 1.5/1. Under these conditions, the amount of DNA protected against micrococcal nuclease (35–40%) agreed with the average number of nucleosomes (9–14) per SV40 DNA molecule. It is probable that neither the histones nor HMG1 were in a state comparable with that required for assembly of nucleosomes *in vivo*. For example, histone acetylation seems to be required for nucleosome assembly (Ruiz-Carillo *et al.*, 1975; Jackson *et al.*, 1976). HMG1 also undergoes *in vivo* post-synthetic modifications such as acetylation, methylation and ADP ribosylation (Allfrey, 1982) any or all of which may be important for correct assembly both *in vitro* and *in vivo*. The physicochemical structure of DNA used in these *in vitro* experiments was also different from its state *in vivo* (protein association, superhelicity, chemical modifications). In the reconstitution experiments described here, the best extent of assembly was obtained with DNA presenting negative supertwists, while covalently closed DNA in a relaxed state was not favorable and required the presence of a topoisomerase I to relax the positive supertwists generated by nucleosome formation. In the latter case, the assembly was 2-fold lower than with supercoiled DNA.

Jackson and Rill (1981) reported that HMG1 and HMG2 (closely related to HMG1) replace histone H1 in nucleosomes released from mouse myeloma nuclei after very slight treatment with micrococcal nuclease. This replacement of H1 by HMG proteins renders nucleosomes soluble under physiological conditions. They proposed that the major function of HMG1 and HMG2 is to replace H1 and to maintain the solubility and accessibility of local chromatin regions.

At physiological ionic strength, HMG1 appears to bind exclusively to single-stranded DNA. This suggests that HMG1 might be included in chromatin structure either by binding to single-stranded DNA regions or by interacting with a free histone surface. Our previous results (Bonne *et al.*, 1979; De Recondo *et al.*, 1980) demonstrated that HMG1 lowered the melting point of poly[d(A-T)] and stimulated rat liver DNA polymerases *in vitro* only when it was isolated from regenerating rat liver. HMG1 isolated from normal rat liver was inactive. This 'melting activity' of HMG1 isolated from dividing cells may be related to its role in facilitating the formation of, and transiently stabilizing, single-stranded DNA whenever the need for this conformation arises during replication or other processes such as transcription. In this study, we show that HMG1 is also able to rapidly assemble

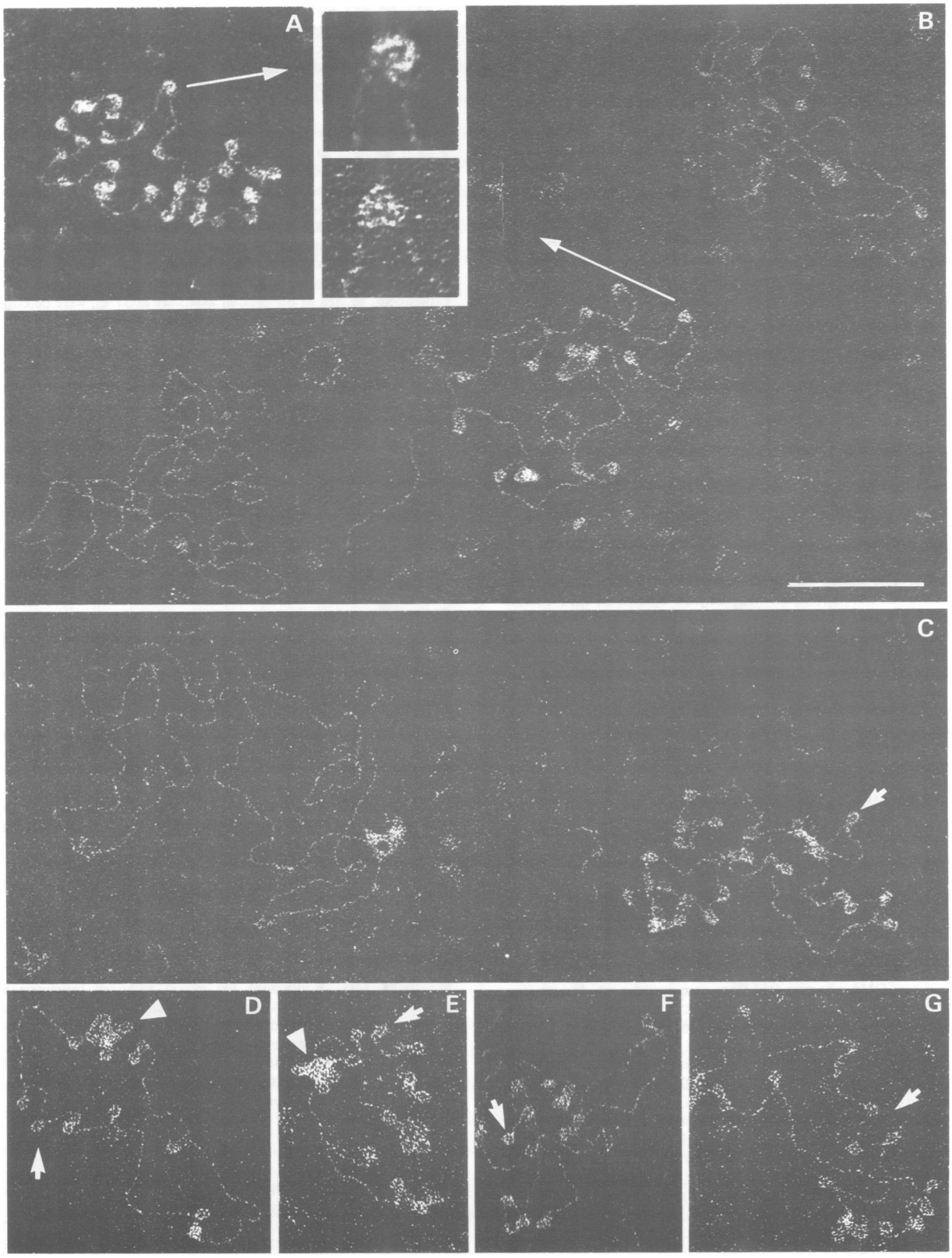

Fig. 4. Electron microscopy study of reconstituted nucleosomes. **(A)** Example of a native SV40 minichromosome isolated from infected cells as described in Materials and methods. This viral chromatin displays 26 nucleosomes evenly spaced. **(B, C, D, E, F, G)** Histones, HMG1 protein and SV40 DNA I were combined as indicated in Figure 3 and the assembly product was spread to reveal a mixture of twisted or relaxed (*) naked DNA and complexes with a variable number (1 – 22) of bead particles. The perinucleosomal location of DNA is clearly visualized by uranyl acetate staining and many particles display a central granule (small arrows) also visible in the native core particles. The structural similarity between native and reconstituted particles is demonstrated at high magnification (x 800 000) in the inserts indicated by large arrows. In the reconstituted complexes, the nucleosomes were generally evenly spaced **(F,G)** but some of them were densely packed **(D,E,** arrowhead). Bar = 100 nm. (*) SV40 DNA I was contaminated by a small amount of DNA II.

histones and DNA in nucleosomes. An assembly factor with a specific affinity for single-stranded DNA on domains distinct from the domain having assembly activity almost certainly plays an important role in DNA replication in eukaryotes. At the replication fork, HMG1 could in some way stabilize single-stranded DNA regions, allowing the DNA polymerase to replicate and, in addition, mediate the rapid, correct assembly of new histones with DNA. Moreover, it was found that the nucleosomal structure of old histones is maintained during replication *in vivo* (for review see De Pamphilis and Wassarman, 1980). Different models have been proposed for nucleosome segregation during genetic readout (transcription and replication), in which histone octamers become transiently bound to single-stranded DNA (Palter *et al.*, 1979). In such processes, HMG1 would appear to be a factor which promotes such transfer during unwinding of the DNA helix.

Materials and methods

Proteins and DNA

HMG1 was purified from regenerating rat liver as previously described (Duguet and De Recondo, 1978).

For preparation of histones, rat liver nuclei were prepared according to the method of Chauveau *et al.* (1957). Nuclei at 60 A_{260} units/ml in 0.25 M sucrose, 10 mM Tris-HCl (pH 8.0), 1 mM $CaCl_2$ were digested with micrococcal nuclease (Worthington) at 150 units/ml for 15 min at 37°C. Digested nuclei were pelleted at 4°C and lysed by resuspension in 10 mM Tris (pH 8), 0.2 mM EDTA. The digest was made up to 10 mM EDTA and, after clarification, the soluble chromatin was dialyzed against 50 mM sodium phosphate pH 7.5, 0.1 mM phenylmethylsulfonyl fluoride, 0.55 M NaCl and applied to hydroxyapatite (Biogel DNA grade), which had been equilibrated with the same buffer. Histone H1 was eluted in the void volume. H2A, H2B, H3 and H4 were eluted by raising the salt concentration to 2.2 M, and concentrated under air pressure through Sartorius microcollodion bags. The concentrated histones were then dialyzed against 2.0 M NaCl, 10 mM Tris-HCl (pH 8.0), and stored at −20°C. Analysis by SDS-polyacrylamide gel electrophoresis showed that the core histones were present in equal amounts and that no H1 was present.

Rat liver topoisomerase I was purified according to Champoux and MacConaughy (1976) from nuclei through a phosphocellulose pool (fraction III). This preparation was a gift of G.Mirambeau and M.Duguet.

Protein markers: *Escherichia coli* β galactosidase was obtained from Sigma; phosphorylase a and bovine serum albumin from Boehringer Mannheim.

Tritium-labelled SV40 supercoiled DNA, prepared according to the Hirt method (1967) was from T.Soussi and M.Philippe. Unlabelled SV40 DNA I was from T.Soussi. Relaxed, covalently closed SV40 DNA (DNA Ir) used for supercoiling assays was prepared by 20 min incubation with topoisomerase I in the conditions described by Champoux and MacConaughy (1976).

Nucleosome core DNA was prepared from core particles which were fractionated from a micrococcal nuclease digest of rat liver nuclei, on a 5–20% sucrose gradient, centrifuged at 27 000 r.p.m. for 20 h at 4°C in a SW27 rotor.

For isolation and partial purification of SV40 minichromosomes, MA 134 cells were labelled with [^{3}H]methyl thymidine at 30 h post-infection. At 42 h, the nucleoprotein complexes were extracted as described by Varshavsky *et al.* (1977). The samples were layered on 5–30% sucrose gradients in 0.15 M ammonium acetate, 10 mM triethanolamine HCl (pH 7.5), 1 mM EDTA. They were centrifuged in a SW 41 rotor at 40 000 r.p.m. for 80 min at 4°C. The most concentrated fractions were pooled. Protein concentrations were determined by the micromethod of Schaffner and Weissmann (1973), using bovine serum albumin as standard.

DNA concentrations were determined spectrophotometrically using: 1 mg/ml = 20 A_{260} units.

Protein cross-linking with dimethyl-suberimidate

Proteins were in a 0.15 M NaCl, 10 mM Tris pH 7.5 buffer. Before addition of dimethyl-suberimidate, the solution was adjusted to 50 mM triethanolamine hydrochloride, pH 9.5, by addition of concentrated triethanolamine, pH 9.5 (Davies and Stark, 1970). Immediately before use, dimethyl-suberimidate was dissolved at 25 mg/ml in the same buffer. Protein concentrations were 0.1–0.4 mg/ml, and the dimethyl-suberimidate concentration was 10 mg/ml by four successive additions of 2.5 mg/ml of the reagent at a 1 h interval.

The reaction was stopped by addition of 1.5% SDS in the presence of 0.7 M 2-mercaptoethanol, 80 mM Tris-HCl, pH 6.8, 0.12% bromophenol blue, and 10% glycerol. The resulting cross-linking products were denatured 3 min at 100°C according to the procedure of Laemmli (1970) and then analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Supercoiling assay

The assembly was performed by dilution of concentrated histones in an HMG1 solution to a final concentration of 0.15 M NaCl, pH 7.5, at the weight ratio indicated in the legends to the figures. After 10 min at 37°C, 1 μg of SV40 DNA Ir and topoisomerase I were added. After 30 min incubation at 37°C, the reaction was stopped by addition of 0.5% SDS (final concentration) and proteinase K (200 μg/ml) and incubated 1 h at 37°C. Sample buffer (15% sucrose, 0.1% bromophenol blue, 0.1% Xylene cyanol) was added and the samples were electrophoresed on 1% agarose gels in the conditions previously described (Duguet *et al.*, 1981). The amount of topoisomerase I added in each experiment, as well as the time of assembly at 37°C, were determined to obtain relaxation of DNA without nucleosome assembly.

Micrococcal nuclease digestion

Assembly of nucleosome cores was achieved under the conditions described above in the supercoiled assay. Modifications in the standard procedure are indicated in the legends to the figures. Samples were made to 1 mM $CaCl_2$ and micrococcal nuclease was added to 1 U/μg DNA. The reaction mixture was incubated 5 min at 37°C, time allowing a total digestion of naked DNA, and the digestion was terminated by addition of EDTA to 15 mM and cooling the reaction mixture to 4°C. The DNA fragments were prepared for electrophoresis as described above for the supercoiling assay. The samples were electrophoresed in an 8% acrylamide slab gel in Tris/borate/EDTA buffer at 10 V/cm for 3 h.

In order to measure the amount of nuclease-resistant product, aliquot fractions were precipitated with 0.6 ml TCA 5%, onto Whatman GF/C glass fiber filters. Filters were washed by TCA 5% and dried before being counted.

Electron microscopy

The specimens were diluted to a final concentration of 0.5–1.0 μg/ml of DNA with double-distilled water and 5 μl were absorbed for 90 s to electron microscopy grids coated with a positively charged carbon film (Dubochet *et al.*, 1971). For dark-field electron microscopy, 600-mesh grids were covered with a very thin carbon film. Specimens were stained with four drops of 2% (w/v) aqueous uranyl acetate. The last drop of stain was rapidly and completely eliminated with filter paper. Dark field observations were carried out with the Philips 400 electron microscope at 100 kv with a 30 μm objective aperture.

The dark field was obtained by the beam tilt method. The micrographs were recorded on Kodak electron image film 4489 at a direct magnification of 36 000–80 000. All measurements were carried out on positive prints of dark field preparations at a final magnification of 240 000–800 000. The length measurements were performed with a Hewlett Packard Graphic digitizer. Diameters of the nucleosomes were measured using a precision magnifier (Nachet, Paris, France).

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