

HMf, a Histone-Related Protein from the Hyperthermophilic Archaeon *Methanothermus fervidus*, Binds Preferentially to DNA Containing Phased Tracts of Adenines

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HMf, a histone-related protein from *Methanothermus fervidus*, was found to bind preferentially to a DNA that is intrinsically bent as a result of the presence of phased oligo(dA) tracts. The intergenic regions in *M. fervidus* DNA are A+T rich and frequently contain oligo(dA) tracts, some of which may have the size and phasing required to create a net bending in one direction. The binding of HMf to bent DNA could play a direct role in gene expression and stabilization of the genome of this organism.

Methanothermus fervidus grows optimally above 80°C, yet its genomic DNA has an overall A+T content of 67% (24) and contains intergenic regions which are greater than 80% A+T (7, 17, 28). The structural integrity of its DNA must therefore be maintained by extrinsic factors, such as its high intracellular salt concentration (8) and stabilization by the abundant DNA-binding protein HMf (13, 17). The binding of HMf to DNA in vitro results in the assembly of nucleosome-like structures (NLS) in which the DNA duplex is rendered relatively heat stable (13). HMf binding has been found to inhibit transcription of DNA in vitro (26). These observations raise an important question: if NLS particles are formed in vivo, how is gene expression regulated in *M. fervidus* with a genome that is compacted into these particles?

Nucleosome phasing and exclusion have been shown to be important for the regulation of gene expression in eukaryotes (5, 12). There is now substantial evidence that a variety of factors, including the underlying DNA sequence, can serve to facilitate or inhibit nucleosome assembly along a segment of DNA (22, 25, 27). Sequence-directed nucleosome assembly has been shown to preferentially occur on DNA segments that are intrinsically bent or are isotropically flexible, both features presumably reducing the energy required to wrap DNA about the histone core (2, 19–21). Other sequences such as extended tracts of oligo(dA) inhibit nucleosome assembly (16, 23). Intrinsically bent DNAs are of particular interest since they frequently occur in functionally important regions, including origins of DNA replication and promoters of transcription (reviewed in reference 6).

HTa (1) from *Thermoplasma acidophilum* and HMf from *M. fervidus* (17, 18) have amino acid sequences that indicate a common evolutionary ancestry with eukaryotic histones. In this study, we have examined the binding of HMf to DNA containing intrinsic bends and oligo(dA) tracts. We have taken advantage of a plasmid, pJGC1/svt (Fig. 1), constructed in earlier experiments (10) in which two bent segments were inserted into pBR325 DNA. One segment, from *Crithidia fasciculata*, contains 18 oligo(dA) tracts 4 to 6 nucleotides in length, 16 of which are in phase with the helix (11). This 223-bp segment has been shown by electron microscopy (EM) (3) and gel electrophoretic analysis (11) to

be highly bent. The other bent segment, from the terminus of simian virus 40 (SV40) DNA replication, is also bent, as seen by EM and electrophoretic analysis (10), but less so than the 223-bp element, and it lacks the clear motif of repeating tracts of adenines present in the bent *C. fasciculata* DNA. In this report, we show that there is a marked preference for HMf binding to the 223-bp DNA and a modest preference for binding to the SV40 terminal element.

Following digestion of pJGC1/svt with *Sph*I and *Sal*I, biotin was incorporated at the *Sal*I-cut end, which was then tagged with streptavidin to uniquely identify one end from the other in electron micrographs. HMf was incubated with this DNA at a mass ratio of 1:1 (0.1 µg of DNA:0.1 µg of HMf in 10 µl) for 10 min at 37°C (in a buffer containing 100 mM NaCl, 50 mM Tris-HCl [pH 7.9], and 2 mM NaPO₄), and the samples were then fixed by exposure to 0.6% glutaraldehyde for 10 min at 25°C. As previously reported (17), apparently protein-free DNA with quasi-spherical particles (NLS) associated along their lengths were seen by EM (Fig. 2). The locations of 258 NLS particles along 128 pJGC/svt DNA molecules were mapped (Fig. 3) by projecting the

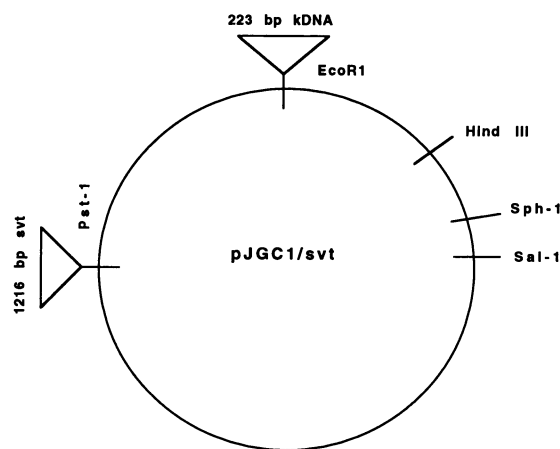


FIG. 1. Restriction map of pJGC1/svt (10). svt, the 1,216-bp segment from SV40 containing the natural terminus of replication; 223-bp kDNA, the highly bent DNA fragment from the kinetoplast minicircles of *C. fasciculata*.

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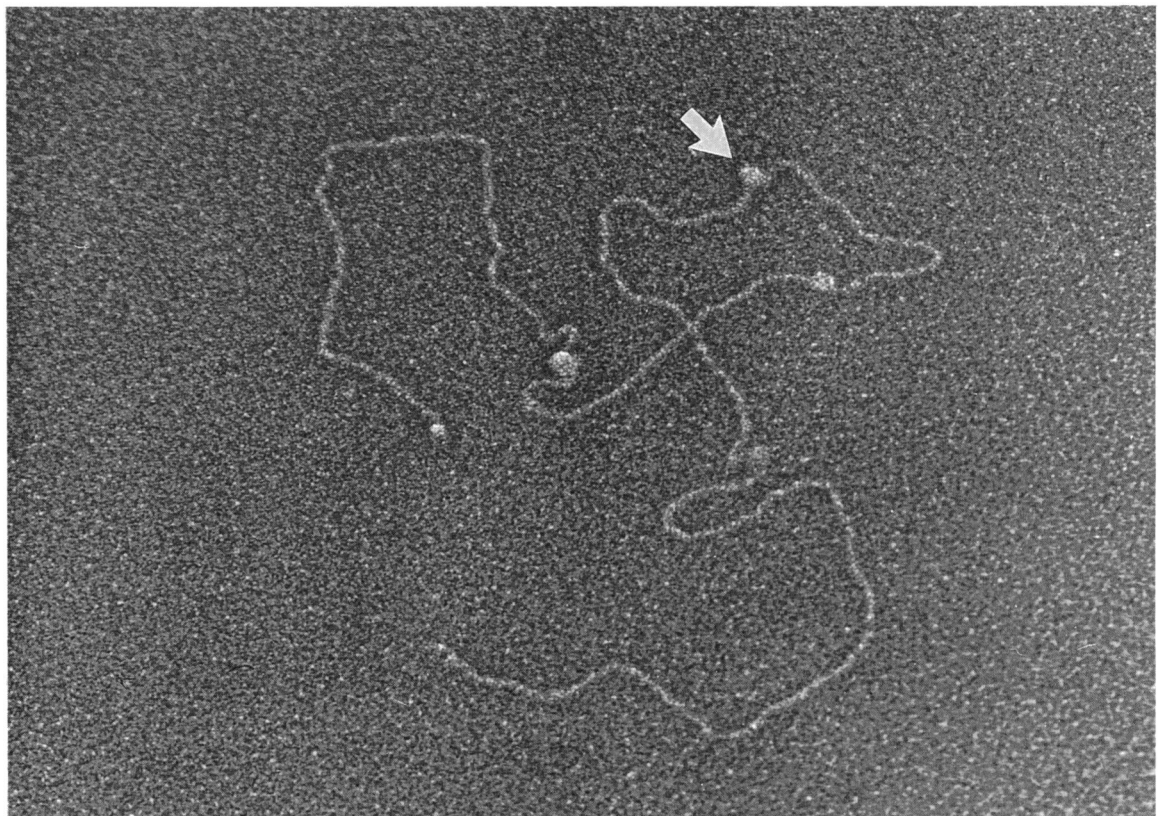
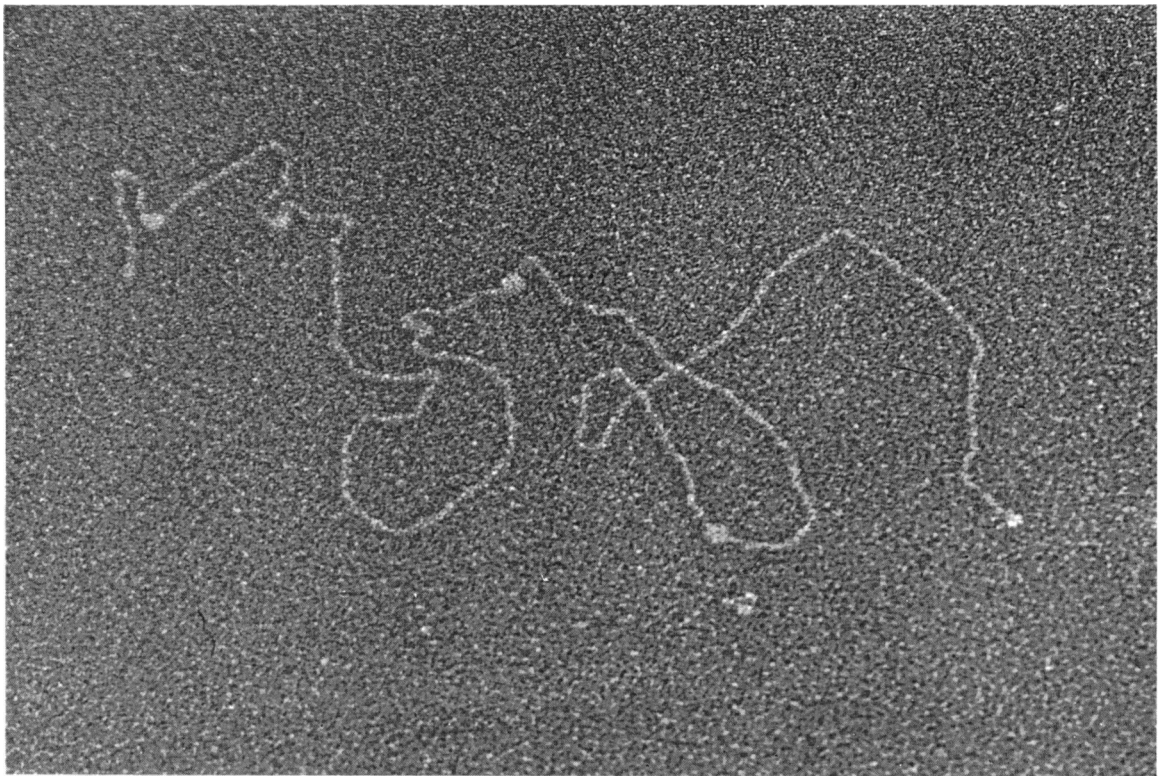


FIG. 2. Electron micrographs of HMf-DNA complexes. Plasmid pJGC1/svt DNA (10) was digested with *SalI* and *SphI*, end labelled with biotin by using biotinylated dCTP (9), and incubated with streptavidin (GIBCO) at a 50× molar excess of protein to DNA ends. HMf was purified as previously described (13) and allowed to bind to DNA at a 1:1 mass ratio. HMf-DNA complexes were fixed by exposure to glutaraldehyde. The samples were then chromatographed over 2-ml columns of Sephacryl S-400 and prepared for EM as described previously (4), including rotary shadow casting with tungsten at 10^{-6} torr (ca. 133.3×10^{-6} Pa). Samples were examined in a Philips 400 EM TLG. The arrow indicates the location of an NLS particle. Bar = 0.1 μ m.

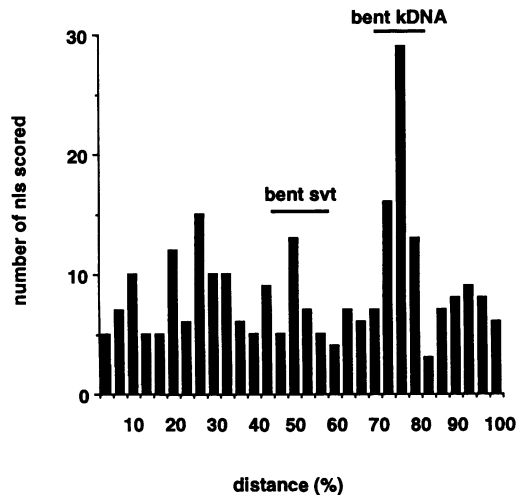


FIG. 3. Location of HMf binding to pJGC1/svt DNA. HMf-DNA complexes were prepared as described in the legend to Fig. 2. The sites at which NLS particles assembled to the linearized plasmid DNA are shown, with locations of the intrinsically bent kinetoplast DNA (kDNA) and SV40 DNA replication terminus (svt) sequences indicated. The total length of the DNA was 7,350 bp, and position 0 was the *SaII* site (see Fig. 1). The position of HMf molecules was determined by measuring the distance from the *SaII* end to each NLS particle.

electron micrographs onto a Summagraphics digitizer coupled to a Compuadd computer programmed with software developed for this purpose. Only those DNAs having one or two particles were analyzed to minimize errors in placement due to the DNA length foreshortening by each NLS particle. As shown (Fig. 3), NLS formation occurred preferentially at the kDNA sequences, with a fourfold enhancement over the average level at nonbent sites within the plasmid. In contrast, the SV40 terminus region assembled NLS particles with only a slightly higher preference than at the basal level. Furthermore, several sites within the parent pBR322 sequences assembled NLSs with efficiency nearly equal to that of the SV40 terminus, and one of these sites (27 to 30% from the *SaII* site) maps near the origin of pBR322, a region known to contain a sequence-directed bend (15).

The ability of HMf to compact DNA was investigated by comparing the overall lengths of HMf-DNA complexes with those of HMf-free DNA. Incubating HMf with linear pGEM3 DNA at a mass ratio of 1:1 (0.15 μg of DNA:0.15 μg of HMf in 20 μl) (Fig. 2) resulted in the assembly of several NLS particles per DNA molecule as seen by EM. DNAs that had either one or two NLS particles were analyzed separately and compared with protein-free DNA (Fig. 4). This analysis demonstrated that one and two NLS particles associated per DNA molecule shortened the length of the complex by 0.04 ± 0.02 and 0.1 ± 0.05 μm , respectively, or about 150 ± 75 bp per NLS particle.

In summary, we found that NLS formation occurred with a strong preference at a sequence in which oligo(dA) tracts are phased to create a strong net bend in one direction and that DNAs with lesser bends assembled NLS particles with a level that appeared to reflect the degree of bending. Further work will be required to distinguish between the contribution of net bending in one direction from the A+T-rich nature of these DNAs as well as the size and phasing of the oligo(dA) tracts to NLS formation. The NLS particles

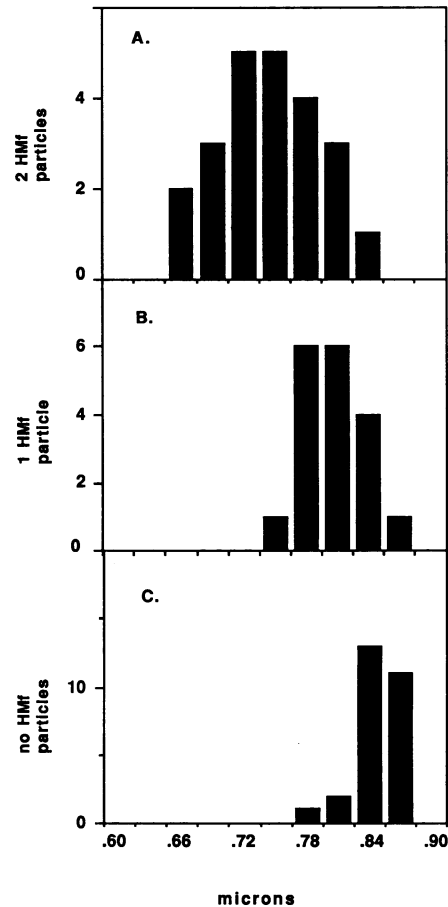


FIG. 4. Linear foreshortening of DNA by HMf. Complexes of *Bam*HI-linearized pGEM3Z+ DNA with HMf were prepared for EM as described in the text. The curvilinear lengths of these complexes containing either one (A) or two (B) NLS particles per DNA were determined by projecting images of electron micrographs onto a Summagraphics digitizer coupled to a Compuadd computer programmed with software developed for this purpose. (C) *Bam*HI-linearized pGEM3Z+ DNA was prepared for EM exactly as described above, with the omission of HMf protein. The curvilinear lengths of protein-free DNA were determined as described above.

were previously calculated to sequester between 90 and 150 bp of DNA (14), a value in good agreement with the direct measurement made here. The intergenic regions of *M. feravidus* are rich in oligo(dA) tracts, some of which will have the size and phasing required to produce a net bending in one direction. The possibility that these sequences (and possibly other intergenic sequences) provide preferential sites for NLS assembly and that this feature in turn is crucial for the stabilization of the *M. feravidus* genome and regulation of its gene expression provides an attractive hypothesis for future study.

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