Functional domains of chlamydial histone H1-like protein

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Chlamydia trachomatis is one of the few prokaryotic organisms known to contain proteins that bear amino acid similarity to eukaryotic histone H1. It is also appreciated that chlamydial histone-like proteins, designated Hc1 and Hc2, can bind DNA and are presumably involved in the condensation of infectious elementary bodies. However, there is no information on either the orientation of Hc1 and Hc2 or the mechanism of their DNA–protein and protein–protein interactions. Whereas the C-terminal domain of Hc1 between amino acids 63 and 125 shows best alignment with sea-urchin histone H1, the N-terminus between amino acids 1 and 62 is highly conserved among various chlamydial species, suggesting a bifunctional role for this unique

protein. In order to delineate the regions responsible for the Hc1 characteristics, we have expressed these two fragments independently in *Escherichia coli* and studied the binding of double-stranded DNA to either whole Hc1 protein or its two termini. Our results support the role of the carboxyl portion in DNA–protein interaction, a function similar to its eukaryotic counterpart. Although this interaction initiates DNA condensation in the absence of the N-terminal domain, it is not sufficient to produce complete compaction. Intra- or inter-molecular protein–protein interactions may be necessary to achieve such an effect.

INTRODUCTION

Chlamydia is an obligate intracellular parasite of eukaryotic cells which has been implicated in a wide spectrum of human diseases [1]. One of the outstanding properties of this organism is its unique developmental cycle [2]. Infection of eukaryotic cells is initiated by attachment of elementary bodies (EBs). Once inside the host cell, chlamydiae undergo morphological and biochemical changes which transform the infectious EBs into proliferative reticulate bodies (RBs). The non-infectious RBs divide by binary fission within cytoplasmic inclusions until they re-differentiate into condensed EBs which are released and are capable of infecting other host cells.

One of the remarkable morphological features that distinguishes RBs from EBs is their DNA structure [3]. In RBs the DNA is loosely packed occupying a major part of the cytosol, thereby resembling other bacteria. EBs, on the other hand, contain a highly electron-dense nucleoid indicative of DNA condensation. Wagar and Stephens [4] reported the presence of two DNA-binding proteins in *Chlamydia* with molecular masses of 18 and 32 kDa. They suggested that these proteins may be involved in DNA condensation during the transition from RB to EB. Subsequently the genes encoding these proteins, which are expressed late in the developmental cycle, have been cloned from *Chlamydia trachomatis* [5–8] and *Chlamydia psittaci* [9]. The 18 kDa protein designated Hc1 exhibits no heterogeneity in its apparent molecular mass among several chlamydial species. Since the terms CTH1 and Hc1 have been coined for the same protein, with designation Hc1 being used by most groups working with this protein, we propose to use the name Hc1 to reduce confusion. In contrast, the 32 kDa protein Hc2, although also found in several chlamydial species, appears to vary in molecular mass and size among species. Both proteins exhibit high lysine content and display similarity to the eukaryotic histone H1. Although both proteins have been implicated in DNA binding

and possible compaction [5,10–13], the intermolecular interaction between Hc1 and Hc2, if any, is not known. It has also been suggested that the range of Hc2 molecular masses observed in different serovars is due to truncated forms of a common protein, since they all display similarity in the N-terminal sequence [14].

In eukaryotes, histone H1 contains three different domains which are claimed to be chemically, structurally and functionally distinguishable [15,16]. The central globular trypsin-resistant G-H1 domain, which is responsible for the specific binding of histone to chromatin, is flanked by the N-H1 domain, which is involved with the correct location of G-H1 on the nucleosome. Finally the C-H1 domain is responsible for DNA condensation. In order to assess possible functional roles for the C- and N-terminal domains of the *C. trachomatis* Hc1, we have expressed in *Escherichia coli* peptides comprising amino acids 2–65 or 68–125 of Hc1. Subsequently, we have studied their ability to polymerize and bind DNA both *in vivo* and *in vitro*. Our results clearly indicate that the C-terminal domain of Hc1 is capable of binding DNA.

MATERIALS AND METHODS

Bacterial strains

C. trachomatis L₂/434/Bu was grown in HeLa 229 cells, and EB was purified as described previously [17]. *E. coli* XL1-Blue (Stratagene) was used as host for Bluescript KS+ [18] and as a general cloning vector. *E. coli* host lysogen BL21 (DE3) [19] was used for the expression of recombinant proteins in pT7-7 [20] under the control of T7 RNA polymerase. Strains BW313 and CJ236 (*dut ung*) were used in the oligonucleotide-directed mutagenesis [21]. *E. coli* were either grown in rich Luria broth or minimal M9 medium, at 37 °C. Cells were made competent as described by Hanahan [22].

Abbreviations used: EB, elementary body; RB, reticulate body; IPTG, isopropyl β -D-thiogalactopyranoside.

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Figure 1 Schematic restriction map and construction in pT7-7 of plasmids pHc1M, pH1W, pH1N and pH1C

All constructions were derived from the original plasmid pCTH1 [8]. Site-directed mutagenesis was used to generate a stop codon between nucleotide positions 307 and 309 and create a new *KspI* restriction site at nucleotide position 311 in the plasmid pHc1M. Histone-specific open reading frame is shown by an open box, and the dark-shaded boxes indicate non-coding regions. The spotted box at the 3' end indicates putative open reading frame 1 of plasmid pCTH1 [8]. The numbers refer to the nucleotide position from the *Bam*HI site of pCTH1. The amino acids and nucleotides encoded by plasmid pT7-7 polylinker are shown in italics. Abbreviations used are: B, *Bam*HI; D, *Dra*I; Ha, *Hae*III; H, *Hind*III; V, *Eco*RV; E, *Eco*RI; K, *Ksp*I.

DNA manipulation

Standard recombinant DNA techniques were used for nucleic acid preparation and analysis [23]. Plasmid DNA was isolated by an alkaline lysis method [24] or boiling method [25]. Radioactive probes were generated by labelling DNA both *in vivo* and *in vitro*. For *in vivo* labelling *E. coli* cultures were grown overnight in the presence of 50 μ Ci/ml [32 P] P_i (300 Ci/mmol; Amersham Corp., Arlington Heights, IL, U.S.A.), and *in vitro* labelling was performed by the random-primer method [26].

All enzymes and reagents used were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.), Fisher Scientific (Edmonton, Alberta, Canada), Boehringer-Mannheim (Laval, Quebec, Canada) or Bethesda Research Laboratory (Life Technologies, Burlington, ON, Canada).

Site-directed mutagenesis

M13 phage containing the wild-type Hc1 structural gene was constructed by subcloning the 1.15 kb *Eco*RI–*Bam*HI fragment from plasmid pCTH1 [8] into M13mp18. Site-specific mutagenesis was carried out (to introduce a stop codon and a new *Ksp*II restriction site) using single-stranded M13 phage DNA containing the Hc1 gene as a template by the method of Kunkel et al. [21]. Oligonucleotide 5'-TAGCCGCGGCTTAGCTT-3' corresponding to nucleotides 319–303 of pCTH1 was used to prime the synthesis of a double-stranded DNA. The histone H1-

like mutant construct designated Hc1M was confirmed by sequence and restriction endonuclease analysis.

Cloning of H1W, H1N and H1C in pT7-7

A 0.4 kb *Hae*III–*Hae*III fragment containing the Hc1 structural gene was made blunt-ended with T4 DNA polymerase and cloned into the *Sma*I site of pT7-7 resulting in plasmid pH1W. The recombinant plasmid is capable of encoding a fusion protein, designated H1W, that includes the first six amino acids of plasmid pT7-7 polylinker along with amino acids 2–125 of Hc1 (Figure 1). A similar construct was generated from the 0.4 kb *Hae*III–*Hae*III fragment of Hc1M resulting in plasmid pH1N which encodes a polypeptide designated H1N, containing amino acids 2–65 of Hc1. In addition, a 0.6 kb *Ksp*II–*Eco*RV fragment of Hc1M was subcloned into the *Bam*HI site of pT7-7. Both the vector and target were blunt-ended with the enzyme T4 DNA polymerase before ligation and transformation. The resulting plasmid, pH1C, encodes a polypeptide designated H1C comprising amino acids 68–125 of Hc1. Figure 1 shows the partial amino acid sequence of the different proteins expressed by each individual clone.

Expression of recombinant proteins

E. coli BL21 (DE3) transformed with pT7-7, pH1W, pH1N or pH1C were grown at 37 °C in Luria-broth medium containing 100 μ g/ml ampicillin. At mid-exponential phase isopropyl β -D-thiogalactopyranoside (IPTG) was added to individual cultures to a final concentration of 1 mM and incubation continued for the desired time. Cultures were harvested by centrifugation at 12000 *g* for 1 min and resuspended in SDS loading buffer.

Preparation of antibodies

E. coli BL21 cells expressing H1C and H1N were resolved by SDS/PAGE. A strip of gel containing the recombinant protein was excised and electroeluted in SDS running buffer. After dialysis of the electroeluted protein, the sample was concentrated using Centricon 3 microconcentrator (Amicon Division, Beverly, MA, U.S.A.). Some 500 μ g of the gel-purified protein emulsified in an equal volume of Freund's adjuvant (Difco Laboratories, Detroit, MI, U.S.A.) was used to immunize female New Zealand White rabbits by subcutaneous injection. Booster shots of the antigen were given every 10 days. After 40 days the rabbits were bled and the serum was stored at –70 °C.

SDS/PAGE and immunoblotting

Total cellular proteins were resolved by SDS/PAGE [27]. Western blots were prepared essentially as described by Towbin et al. [28]. Nitrocellulose membranes were blocked for 30 min in PBS containing 5% skimmed milk. The membranes were incubated at room temperature with monospecific polyclonal antibodies in blocking solution (at a final antibody dilution of 1:1000) followed by three washings for 10 min in PBST (PBS plus 0.1% Tween 20). The immunoblots were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (heavy- and light-chain-specific; Cappel) for 1 h, washed three times in PBST and bound peroxidase was detected by incubating the blots in substrate solution containing 4-chloro-1-naphthol (Bio-Rad Laboratories, Hercules, CA, U.S.A.).

Monospecific antibodies were prepared as described by Olmsted [29]. Essentially whole serovar L₂ EB lysates were resolved by SDS/PAGE and transferred electrophoretically to nitrocellulose. Strips containing 18 kDa Hc1 protein were excised

and incubated with either polyclonal rabbit anti-H1N or anti-H1C antibodies at 1:200 dilution for 2 h. The bound antibodies were eluted with 0.2 M glycine/HCl, pH 2.8, neutralized immediately with 1 M Tris/HCl, pH 8.0, and used in immunoblot analysis.

DNA-binding assay

Assay of *in vitro* DNA-binding activity of the whole cell extract using the Southwestern-blotting technique was carried out by the method of Wagar and Stephens [4]. Each nitrocellulose membrane was probed with 10^7 c.p.m. of radiolabelled DNA probe.

Gel mobility-shift assay

Purified H1W, H1C and H1N polypeptides (ranging from 0.4 to 0.00625 mg) were mixed with 0.5 μ g of recombinant chlamydial plasmid DNA (pCTP1). The reaction volumes were adjusted to 15 μ l with PBS containing 50% glycerol and incubated at 37 °C for 10 min before loading. The complexes were separated on 0.8% (w/v) agarose (Bio-Rad) gels in Tris/acetate buffer, stained with 0.5 μ g/ml ethidium bromide and visualized under UV light. Plasmid pCTP1 was constructed by cloning *Pst*I linearized 7.5 kb *C. trachomatis* plasmid into the *Pst*I site of plasmid pBR322. The authenticity of plasmid pCTP1 was confirmed by Southern-blot analysis and restriction mapping and compared with previously published data [30].

Electron microscopy

A 2 ml *E. coli* broth culture was mixed 1:1 with 2% (v/v) glutaraldehyde/2% (v/v) formaldehyde in 50 mM phosphate buffer, pH 7.4, and immediately centrifuged in a bench-top centrifuge at approx. 1500 rev./min. The supernatant was discarded and the samples were further fixed with 2% glutaraldehyde/2% formaldehyde for 1 h at 4 °C. The samples were centrifuged again and washed twice with cold 50 mM phosphate buffer, dehydrated in a graded series of precooled alcohol and low-temperature-embedded in Lowicryl K4M [31,32] (JBS Supplies, Pointe Claire, Quebec, Canada). Ultrathin sectioning was accomplished by the use of a glass knife and a Reichert-Jung Ultracut ultramicrotome. The sections were placed on copper grids and stained with 5% (w/v) uranyl acetate in methanol and Reynolds lead acetate before examination in a Philips model 410 transmission electron microscope.

RESULTS

Expression of the chlamydial histone(s)

To study the expression of Hc1 in *E. coli*, a 0.4 kb *Hae*III fragment of pCTH1 was cloned into the *Sma*I site of pT7-7. In this experimental strategy six amino acids encoded by pT7-7 polylinker were fused to the Hc1 N-terminus (Figure 1). Expression from the recombinant plasmid designated pH1W containing the Hc1 gene directly downstream of the T7 promoter is dependent on IPTG-inducible T7 polymerase.

To express the N- and C-termini of the Hc1 protein, site-directed mutagenesis was carried out on the wild-type pCTH1 gene (Figure 1). Two mutations were introduced. The first one created a stop codon in the triplet coding for Lys-66, and the second mutation created a new restriction site, *Ksp*I at position 315 (pHc1M; Figure 1). In addition, an internal *Ksp*I-*Eco*RV fragment of pHc1M was inserted into the *Bam*HI site of pT7-7 to express a peptide comprising amino acids 68–125 of Hc1 fused through its N-terminus to nine amino acids encoded by the

plasmid pT7-7. The division of protein Hc1 into N- and C-terminal halves was based on interspecies homology at the N-terminus comprising amino acids 1–66 and an overlap with the C-terminal DNA-binding domain at its C-terminus.

The expression of these recombinant proteins is shown in Figure 2(A). A protein band migrating slightly more slowly than the histone H1-like protein of *L. 2* appears in *E. coli* extracts expressing H1W (Figure 2A, lanes 1 and 2). The difference in observed mobility is due to the five additional amino acids fused at its N-terminus. Similarly, new protein products with estimated molecular masses of 8000 and 14000 respectively were identified from *E. coli* expressing H1N and H1C (Figure 2A, lanes 3 and 4 respectively). Since the two peptides have a similar theoretical molecular mass (7850 Da for H1N and 7100 Da for H1C), the much slower mobility of H1C may be explained in terms of its amino acid composition. The expressed H1C has an estimated isoelectric point of 13.2, compared with 10.1 for H1N. The highly basic nature of H1C is probably responsible for the altered mobility of this protein. It is worth noting that the whole native histone H1-like protein with an estimated molecular mass of 13761 (14193 for recombinant H1W) also migrates to an anomalous position, corresponding to 18 kDa.

Proteins H1C and H1N were subsequently gel-purified and used to immunize rabbits. After immunization the rabbit sera were tested for specificity by Western blotting. As shown in Figures 2(B) and 2(C) (lanes 1 and 2), the antibodies recognize individual polypeptides as well as whole histone H1-like protein from either *C. trachomatis* serovar *L. 2* EBs or *E. coli* expressing H1W. This suggests that the recombinant protein H1W or individual peptides H1N or H1C are antigenically similar to native protein. No antigenic cross-reactivity was observed between preimmune rabbit serum and expressed antigens under similar conditions (results not shown). Rabbit anti-H1N antibodies recognized additional low-molecular-mass immunoreactive bands in cell extracts of *E. coli* expressing H1W that may represent breakdown products of recombinant histone H1-like protein or alternatively premature translation products (Figure 2C, lane 2). However, no such cross-reactivity was observed with anti-H1C antibodies (Figure 2B, lane 2). Evidence that these low-molecular-mass fragments represent Hc1-specific products rather than non-specific interaction of anti-H1N antibodies, was obtained by analysing the [35 S]methionine-labelled products after pulse-labelling *E. coli* harbouring pH1W. Radiolabelled bands similar in mobility to those observed on immunoblots were visualized by autoradiography (results not shown). Rabbit anti-H1N antibodies, however, reacted non-specifically with H1C (Figure 2C, lane 4) but not the converse (Figure 2B, lane 3). This phenomenon cannot be currently explained. We also observed, to our surprise, that nearly 75–80% of radiolabelled H1N polypeptide that was bound after electrophoretic transfer from SDS/PAGE to the nitrocellulose membrane dissociated during the process of immunoblotting and subsequent washings (results not shown). The dissociation of H1N from nitrocellulose blots may explain the weak immunoreactivity observed in some instances.

In vitro DNA binding

E. coli extracts expressing H1W, H1N and H1C were resolved by SDS/PAGE, and transferred to nitrocellulose membranes. These membranes were subsequently incubated with 32 P-labelled DNA that was generated either *in vitro* by the random-primer technique or *in vivo* by extracting DNA from 32 P-labelled *E. coli* cells. DNA prepared by either method was able to bind peptides H1W and H1C. However, no binding signals were observed with H1N

Figure 2 Expression and identification of recombinant gene products

(A) Whole cell extracts of serovar L₂ EB (lane 1), IPTG-induced *E. coli* BL21 cells harbouring plasmid pH1W (lane 2), pH1N (lane 3), pH1C (lane 4) and pT7-7 (lane 5) were resolved by SDS/PAGE and stained with Coomassie Blue. The mobility of low-molecular-mass markers (kDa) is shown on the left. Also shown are the corresponding Western blots probed with polyclonal monospecific anti-H1C antibodies (B) and polyclonal monospecific anti-H1N antibodies (C). The arrowheads indicate the mobility of native and recombinant histone H1 in lanes 1 and 2 respectively. The position of the N-terminal peptide in lane 3 is shown by an asterisk, and a thick arrow indicates the mobility of the C-terminal peptide in lane 4.

Figure 3 Southwestern-blot analysis

Cell extracts of serovar L₂ (lane 1) or IPTG-induced *E. coli* BL21 cells harbouring pT7-7 (lane 2), pH1W (lane 3), pH1N (lane 4) and pH1C (lane 5) were resolved by SDS/PAGE and Western transferred to nylon membrane. The blots were probed with 10^7 c.p.m. of 32 P-labelled pBluescript SK+ DNA (A) or *KpnI*–*Bam*HI fragment of pCT 40–118 encoding chlamydial major outer membrane protein gene [33] (B). The mobility of low-molecular-mass markers (kDa) is shown on the left.

despite longer exposure of the autoradiographs. These results indicate that the DNA-binding capacity of Hc1 is mainly localized to its carboxyl portion. Figure 3 shows the binding of double-stranded DNA to both native and recombinant histone H1-like proteins. No difference in binding pattern between native and recombinant proteins was observed when double-stranded DNA from chlamydial or non-chlamydial sources, namely *E. coli*, was used as probe (compare lanes 1 and 3 of Figures 3A and 3B). However, an additional band of 32 kDa, corresponding to Hc2, appeared in EB extracts only (Figure 3, lane 1).

In vitro DNA–histone H1-like protein interactions

Initial experiments using Southwestern-blot assay failed to demonstrate any binding of DNA to H1N. Whether lack of binding was a reflection of H1N dissociation from nitrocellulose blots during hybridization rather than failure of DNA to interact with H1N was precisely defined by examining the gel mobility shifts of histone(s)–DNA complexes. Agarose-gel electrophoresis of the

Figure 4 Gel mobility-shift assays for analysis of DNA–histone(s) interaction *in vitro*

Recombinant chlamydial plasmid pCTP1 (33.3 mg/ml) was incubated with various amounts of purified H1W (A), H1N (B) or H1C (C). Lanes 1–5 contain 26.5, 13.2, 6.6, 1.6 and 0.4 mg/ml protein respectively. Lane 0 contains DNA alone. The arrow indicates the mobility of supercoiled topoisomeric plasmid DNA. The mobility of λ HindIII DNA markers represented in kb is shown on the right.

complexes (DNA–protein aggregates) revealed mobility shifts dependent on the concentration of histone or its two termini (Figure 4). At a concentration of 26.5 mg/ml, histone H1W retained whole DNA in agarose gel wells, and a significant portion was still retained even at 13.2 mg/ml (Figure 4A, lanes 1 and 2). No visible difference in mobility was observed between the controls without protein and samples incubated with 6.6 mg/ml or less histone H1W (Figure 4A, lanes 0, 4 and 5). In contrast, very little DNA was retained in agarose wells after incubation with 26.5 mg/ml H1N (Figure 4B, lane 1). Even higher concentrations of H1N failed to retain DNA in agarose wells (results not shown). These results suggest a very low affinity of H1N for DNA. H1C, however, retained DNA in the wells at a concentration as low as 6.6 mg/ml, further supporting its role in protein–DNA interaction (Figure 4C, lane 3). Changes in the mobility of supercoiled plasmid topoisomer were also apparent with lower H1W and H1C concentrations.

Figure 5 Transmission electron micrographs of *E. coli* cultures encoding the polypeptides Hc1 (A and C), H1N (E) and H1C (B and D) along with control cells harbouring pT7-7 (F)

Arrowheads indicate intermediate DNA-condensation pattern. Bar 1.0 μ m.

Electron microscopy of *E. coli*-induced cultures

Transmission electron microscopy was used to study the role of histone H1-like protein and its terminal polypeptides in DNA condensation. As shown in Figures 5A and 5C, *E. coli* expressing the H1W reveal a highly electron-dense particle in the middle of the cells, whereas cells expressing H1N are not significantly different from controls (compare Figure 5E with Figure 5F). However, an intermediate condensation comprising up to three small not very electron-dense particles is detected in cells expressing the peptide H1C (Figures 5B and 5D), although the degree of condensation observed in cells expressing the whole protein is never achieved. A more detailed examination of cells expressing H1W (Figure 5A) also revealed some intermediate DNA-condensation patterns similar to the one observed for cells expressing H1C (Figure 5B).

DISCUSSION

The existence of two different eukaryotic histone H1-like proteins in *Chlamydia* has been reported from several laboratories [4–9,12,14]. One of these histone-like proteins, with an observed molecular mass of 18 kDa, is conserved among all *C. trachomatis* serovars, whereas the second exhibits variable molecular mass (25–32 kDa) depending on the serovar. Preliminary studies based on deduced sequence analysis and similarity to eukaryotic histone H1 suggest that these proteins, which are expressed late in the chlamydial developmental cycle, may well be involved in the DNA condensation observed when RBs transform into EBs.

Because of the absence of recombinant DNA techniques in *Chlamydia*, the function of these genes has been studied in *E. coli* [5,10,11]. Recently, two groups have reported the expression of 18 kDa Hc1 in *E. coli* [5,12]. Their results suggest that Hc1 alone is capable of forming complexes with *E. coli* DNA, yielding large aggregates that finally produce condensation of the chromosomal DNA [5,10–13]. We have studied the structure–function relationship of chlamydial Hc1 with the aim of delineating the functional domains of this protein.

Comparison of the Hc1 from *C. trachomatis* and *C. psittaci* reveals 87% identity in the first 66 amino acids whereas the C-terminus shares only 25% identity between the two species [9]. Nonetheless the C-terminal portion in both chlamydial species displays greater identity with eukaryotic histone H1 than does the N-terminus. Therefore, on the basis of sequence comparisons, we can distinguish two different regions in the chlamydial 18 kDa Hc1 protein: the N-terminal half is a *Chlamydia*-specific region whereas the C-terminal portion appears to be common to all organisms possessing histone H1-like protein. In order to characterize the different functional domains we have expressed in *E. coli* proteins containing the first 64 amino acids of the N-terminus or last 58 amino acids of the C-terminus, as well as the whole protein H1W. Expression of the whole protein has been reported to be toxic for *E. coli* [6]; for this reason we have expressed the protein as well as the derived peptides under the control of T7 polymerase in *E. coli* strain BL21 (DE3), which expresses the polymerase in the presence of IPTG. The amount of expression of the C-terminal and N-terminal polypeptides in this system was three to four times greater than that of the whole histone H1-like protein. Furthermore immunoblot analysis suggests that recombinant H1W is either degraded from its C-terminus or is susceptible to premature translation *in vivo* whereas its truncated polypeptides remain intact.

Our data unequivocally support the DNA-binding role for the C-terminus of Hc1. This binding capacity is exhibited both *in vivo* and *in vitro*. Southwestern-blotting experiments have shown that the binding specificity of double-stranded DNA from either *E. coli* or *C. trachomatis* is similar for both H1W and H1C. These observations are in full agreement with published work showing the binding of DNA to whole histone H1-like protein *in vitro* [4,13]. However, failure to observe any DNA–H1N interactions by Southwestern blotting may be due to either very low level interactions which are below detection levels or the loss of protein during hybridization, or a combination of both. The possibility that the basic nature and/or small size may account for the dissociation of bound H1N from nitrocellulose membranes was ruled out on the basis of the H1C DNA interaction. H1C is more basic in nature (pI 13.2) than H1N (pI 10.1) and has similar theoretical molecular mass. Weak interactions were clearly demonstrated between H1N and DNA compared with either H1W or H1C by mobility-shift experiments also. Lower concentrations of H1W (13.2 mg/ml) and H1C (6.6 mg/ml) were able to retard the mobility of DNA complexes when compared with H1N, which barely retarded the mobility of plasmid DNA at all at a concentration of 53 mg/ml. Even at this concentration very little DNA was retained in the well. Previous reports have also shown a retention of histone H1-like protein–DNA complexes in agarose wells [11,13]. More interesting, however, is the observation that Hc1 preferentially bound and altered the mobility of supercoiled DNA. Further, electron microscopy has confirmed previous results showing condensation of *E. coli* chromosomal DNA by chlamydial Hc1 [10]. However, cells expressing the N-terminal half of the histone-like molecule have a morphology similar to non-expressing cells, whereas expression of the carboxyl portion produces an in-

intermediate effect with faint condensation centres but no electron-dense nucleoid. Detailed examination of cultures expressing whole protein shows that, in addition to cells with a highly condensed nucleoid, some cells have looser condensation, similar to those observed in *E. coli* expressing the H1C. This observation may be explained by assuming that the histone binds to DNA mainly through its carboxyl portion as the result of charge interaction which neutralizes the negatively charged DNA, and this is followed by protein-protein cross-linking.

The general assignment of functional domains for the chlamydial histone H1-like protein appears to be similar to the model for eukaryotic histones although clear differences have been observed. In the case of the C-terminus, functional similarity is high since the DNA-binding properties of the chlamydial histone H1-like protein closely resemble those of its eukaryotic counterpart. However, the characteristics of the N-terminal portion of Hc1 do not appear to be so closely related to the eukaryotic N-H1 domain since no nucleosome organization has been detected in *Chlamydia* DNA. Eukaryotic N-H1 plays a role in the correct location of the globular G-H1 domain on the nucleosomes and appears to be responsible for the microheterogeneity of the different H1 variants [34].

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