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Histonelike Proteins of Bacteria

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INTRODUCTION

All higher organisms contain small, basic, abundant, deoxyribonucleic acid (DNA)-binding proteins called histones. These proteins are highly conserved in terms of primary structure, and they are responsible for compacting DNA into nucleosomes (for review, see reference 88). Bacteria contain proteins, termed histonelike, that share some properties with eucaryotic histones. Unlike eucaryotic histones, the bacterial histonelike proteins have not been shown to interact as a unit with DNA to form complexes analogous to nucleosomes, and it has been difficult to develop a definition that applies to all of the proteins considered by various authors to be histonelike. The many similarities between the protein called HU and eucaryotic histones first led to the idea that bacteria contain histonelike proteins (127). HU is a small, basic, abundant, DNA-binding protein capable of wrapping DNA, and its primary structure is highly conserved among bacterial species. We also consider the protein called IHF to be histonelike because it can wrap DNA and because it has considerable amino acid sequence homology with HU. IHF is a host factor that assists the bacteriophage lambda Int protein in promoting site-specific recombination (100). Three other proteins can in some ways be considered histonelike. They are small and abundant, and some are basic. Of these, protein H is the most histonelike; it has an amino acid composition similar to eucaryotic histone H2A (59). Another small, abundant protein is called H1 (18). This neutral protein appears capable of compacting DNA. The third protein is the product of the *firA* gene; this basic protein is thought to be involved in transcription (80). General features of these five proteins are summarized in Table 1.

One of the more exciting developments in this field has been the merger of studies of histonelike proteins and site-specific recombination. The primary structure of a factor (IHF) involved in site-specific recombination is related to that of the prototype histonelike protein HU, and, at least in vitro HU participates in site-specific recombination. Below we review the properties of HU, IHF, H, H1, and FirA with emphasis on their potential roles in bacterial chromatin structure and nucleotide sequence recognition.

The study of bacterial histonelike proteins developed from a number of directions. In the case of the protein known as HU, the first line of investigation involved a search for factors stimulating transcription. Attention focused on proteins eluting from DNA cellulose at relatively high salt concentration, and an abundant low-molecular-weight protein was discovered (127). This protein has an unusual amino acid composition for an *E. coli* protein, and after comparison with many other proteins, it was realized that the amino acid composition of HU resembles that of the eucaryotic histone H2B (127). (HU was first isolated from *Escherichia coli* strain U93 (ribonuclease negative) and was called factor U. The letter H was added when it became clear that the protein shared many characteristics with eucaryotic histones.) A variety of studies were then stimulated by the possibility that HU might be a major component of bacterial chromatin. In an unrelated series of experiments, two small basic proteins, NS1 and NS2, were found to contaminate preparations of ribosomes (144). NS1 and NS2 correspond to the two

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TABLE 1. General properties of histones and histonelike proteins

Protein type	Involved in DNA wrapping	Approx mol wt	Approx bp of DNA per protein monomer	Conserved primary structure
H2A, H2b, H3, H4[a]	Yes	11,000–15,000	100	Yes
HU	Yes	9,500	88–140[b]	Yes
IHF (α, β)	Yes	10,000–11,000	ND[c]	ND
H	ND	28,000	70[b]	ND
H1	ND	15,500	580[b]	ND
FirA	ND	17,000	440[b]	ND

[a] Eucaryotic histones.
[b] Calculation based on copies of protein per cell and on 2 genome equivalents of DNA (8.8×10^6 bp) per cell.
[c] ND, Not determined.

subunits of HU (129, 144). A third line of study was similar to the first, and it produced a protein called HD (4). HD appears to be identical to HU and NS (the collective term for NS1 and NS2), at least by immunological cross-reactivity (129, 144) and by the identity of the first four N-terminal amino acids (74).

As expected, these converging lines of study created nomenclature problems. In this review we hope to clarify relationships among the histonelike proteins; our nomenclature suggestions, along with synonyms, are listed in Table 2. We have preserved the term HU for the prototype protein from *E. coli* because HU was used in the first description of the protein, it has widespread usage, and it is more readily recognized by nonspecialists than the numerical designations subsequently proposed (40). The native HU protein isolated from *E. coli* is a heterotypic dimer (αβ) composed of two closely related subunits (129). The two subunits, termed HU-α and HU-β, can be separated by acid urea-Triton gel electrophoresis, and they are identical to NS2 and NS1, respectively (129). HU has also been termed DBPII (40), and the subunits HU-α (NS2) and HU-β (NS1) have the alternative designations HU2 and HU1, respectively. Having four names has created confusion and errors. Since the subunits of the related protein, integration host factor, are designated IHF-α and IHF-β, we advocate uniform adoption of the parallel nomenclature HU-α and HU-β. HU-α (corresponding to NS2 and HU2) would be encoded by the gene called *hupA* and HU-β (corresponding to NS1 and HU1) would be encoded by the gene called *hupB*, as adopted by Imamoto and his colleagues (62a). For additional clarity in this review, we have included the terms NS, NS1, NS2, and HD in parentheses in the text when discussing experiments with HU where the alternate designations were used in the original descriptions. For species other than *E. coli*, three-letter acronyms are becoming popular for HU-like proteins, and we use them here. In this scheme, the letter H is followed by the first letters of the genus and species name, respectively, for each organism from which the protein was isolated.

At the same time that histonelike proteins were discovered in eubacteria, similar studies were being carried out with a species of archaebacterium, *Thermoplasma acidophilum* (132). Phylogenetically, archaebacteria appear to fall between procaryotes and eucaryotes, since some of their biochemical properties are characteristic of eubacteria while others are characteristic of higher cells. The histonelike protein from *T. acidophilum* reflects this intermediate phylogenetic position: its amino acid sequence exhibits homologies with HU as well as homologies with the eucaryotic histones H2A and H3 (135). Since the study of the archaebacterial protein may lead to some important insights about bacterial histonelike proteins, we have included a brief description of the properties of this protein.

Similarities between HU and eucaryotic histones suggest that DNA packaging in eucaryotic and procaryotic organisms may share common features. However, the two types of organism are strikingly different in terms of how easily their chromatin can be isolated and studied: we still have no good way to isolate chromatin from bacteria. As a result, we cannot say with certainty which proteins, if any, are instrumental in packaging bacterial DNA. Our working hypothesis is that some, if not all, of the bacterial histonelike proteins participate in DNA packaging; consequently, we review bacterial chromatin structure as well as the biochemistry and genetics of the histonelike proteins. For earlier reviews, readers are referred to Rouviere-Yaniv (124), Geider and Hoffmann-Berling (40), Pettijohn (108), Gualerzi et al. (53), and Dijk et al. (22).

BIOCHEMICAL PROPERTIES OF HISTONELIKE PROTEINS

HU Proteins

HU was isolated on the basis of its affinity for DNA, and when obtained from *E. coli*, HU consists of a pair of small, basic proteins having similar amino acid sequences (Fig. 1). HU elutes from DNA cellulose at about 0.4 M NaCl (127), suggesting a moderate affinity of HU for DNA. The strength of binding to DNA cellulose varies with the species from which HU proteins are isolated. For example, HRm from *Rhizobium* spp. and HBs from *Bacillus* spp. are eluted from DNA cellulose by lower salt concentration than is HU. HTa from *T. acidophilum* requires higher salt concentration for elution. The physiological significance of these differences is not known. Early studies also showed that HU is heat stable; solutions containing the protein can be heated to 100°C in 0.4 M NaCl; when cooled, the protein regains its ability to bind DNA (127).

HU binds to both single-stranded and double-stranded DNA as well as to ribonucleic acid (RNA) (HD [4, 57, 159]). Binding to ribosomal RNA probably accounts for the association of the protein with ribosomes in cell extracts (57). It has been reported that the affinity of the protein for single-

TABLE 2. Nomenclature of HU-like proteins

Bacterial species	Protein	Gene
Escherichia coli	HU (NS, HD, DBPII)	NA[a]
	HU-α (HU2, NS2)	*hupA*
	HU-β (HU1, NS1)	*hupB*
	IHF-α	*himA*
	IHF-β	*hip* (*himD*)
Bacillus stearothermophilus	HBs	ND[b]
Pseudomonas aeroginosa	HPa	ND
Rhizobium meliloti	HRm	ND
Clostridium pasteurianum	HCp	ND
Anabaena sp.	HAn	ND
Thermoplasma acidophilum	HTa	ND
Bacillus subtilis phage SPO1	TF1	Mapped[c]

[a] NA, Not applicable.
[b] ND, Not determined.
[c] The gene encoding TF1 is located between coordinates 101.5 and 102.5 on the SPO1 physical map (50).

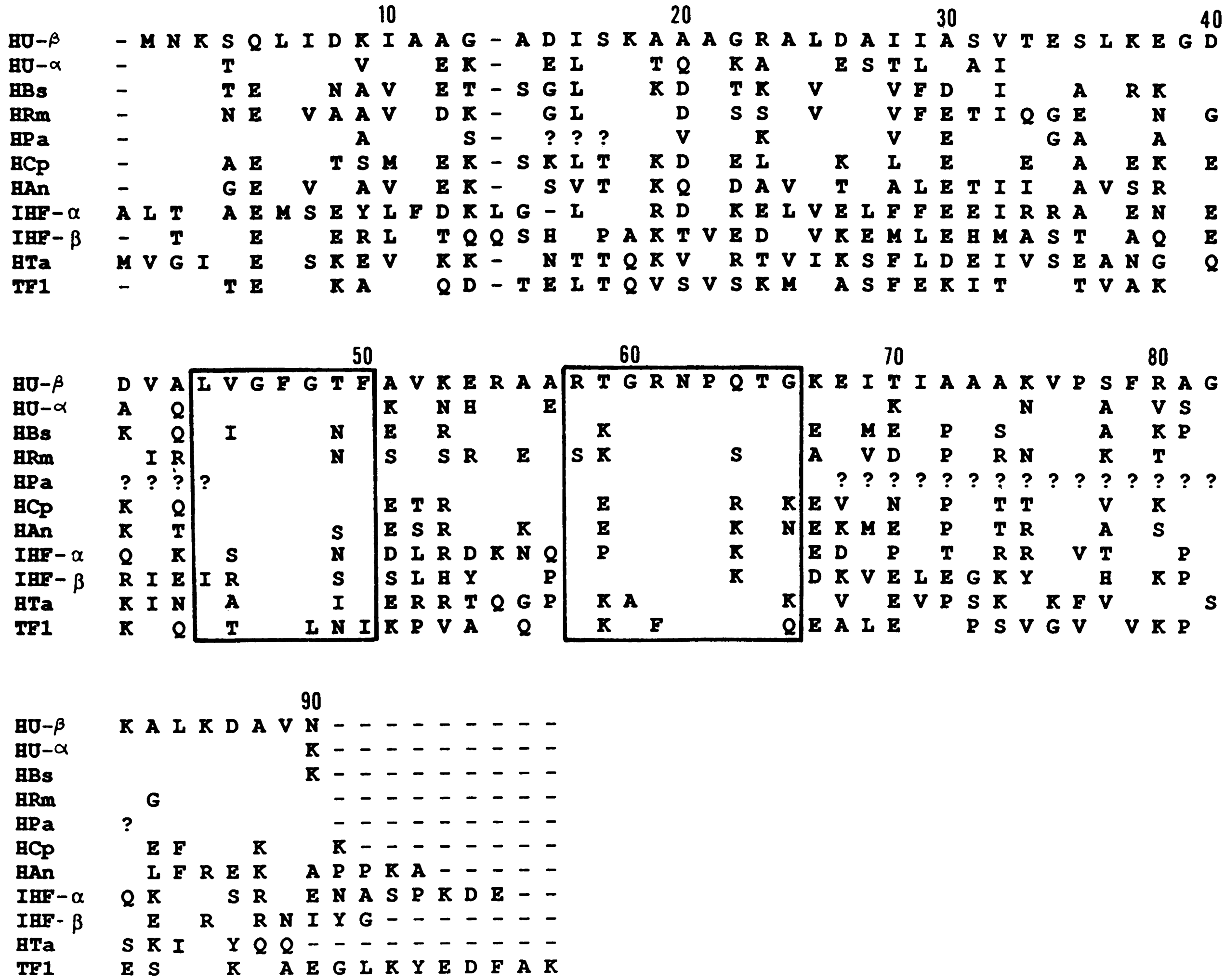

FIG. 1. Amino acid sequence comparison for bacterial HU-like proteins. The amino acid residues are listed according to a one-letter code for HU and HU-like proteins from a number of organisms. Only the sequence for HU-β (HU-B, HU1) of *E. coli* (top line) is listed in its entirety. For other proteins letters are inserted only when the residue differs from that found in HU-β. Hyphens indicate gaps in the sequence to improve the alignment of amino acids. Two regions are boxed to call attention to particularly high levels of homology. The bacterial species from which the proteins were obtained are abbreviated as follows: HU-β, *E. coli* (73, 90); HU-α, *E. coli* (73, 90); HBs, *B. stearothermophilus* (145); HRm, *R. meliloti* (71); HPa, *P. aeruginosa* (56); HCp, *Clostridium pasteurianum* (145); HAn, *Anabaena* sp. (Nagaraja and Haselkorn, cited in reference 33); IHF-α, *E. coli* (89, 92); IHF-β, *E. coli* (33); HTa, *T. acidophilum* (21); TF1, *B. subtilis* bacteriophage SPO1 (50).

stranded DNA is higher than for double-stranded DNA (39, 57); however, this observation has not been reconciled with the finding that HU stabilizes double-stranded DNA against thermal denaturation. For example, at a protein/DNA ratio of 1 and a sodium ion concentration of about 10^{-3} M, the melting temperature of *E. coli* DNA is raised by more than 20°C (128).

In solution, native HU is a heterotypic dimer. Its apparent molecular weight from gel filtration measurements is about 20,000. This is approximately twice the molecular weight (9,500) of the monomer. In addition, cross-linking studies produce a particle having an apparent molecular weight of 20,000 as determined by gel electrophoresis in the presence of ionic detergents (127). Phosphocellulose chromatography of purified HU separates the preparation into three fractions, each containing HU dimers when assayed by gel filtration or by gel electrophoresis after cross-linking (129). Analysis of the protein composition of each fraction revealed that the major fraction, which represents at least 90% of the total protein, contains both HU-α and HU-β (the two subunits, HU-α and HU-β, can be separated by gel electrophoresis [129]). The two minor fractions, which flank the major one, contain only HU-α or HU-β. Thus it appears that most of the native HU protein isolated from *E. coli* exists in solution as a heterotypic dimer (125), and it is likely that HU also functions as a heterotypic dimer (60a). Subsequent biophysical studies indicated that the heterotypic dimer is more stable than the homotypic ones when renaturation following thermal denaturation is monitored (106). In these studies the homodimer of HU-β (NS1) failed to renature as readily as the heterodimer or the homodimer of HU-α (NS2). It is important to note that many species of eubacteria contain only a single type of HU subunit (Fig. 1), and in these cases HU is probably a homodimer. Thus caution must be exer-

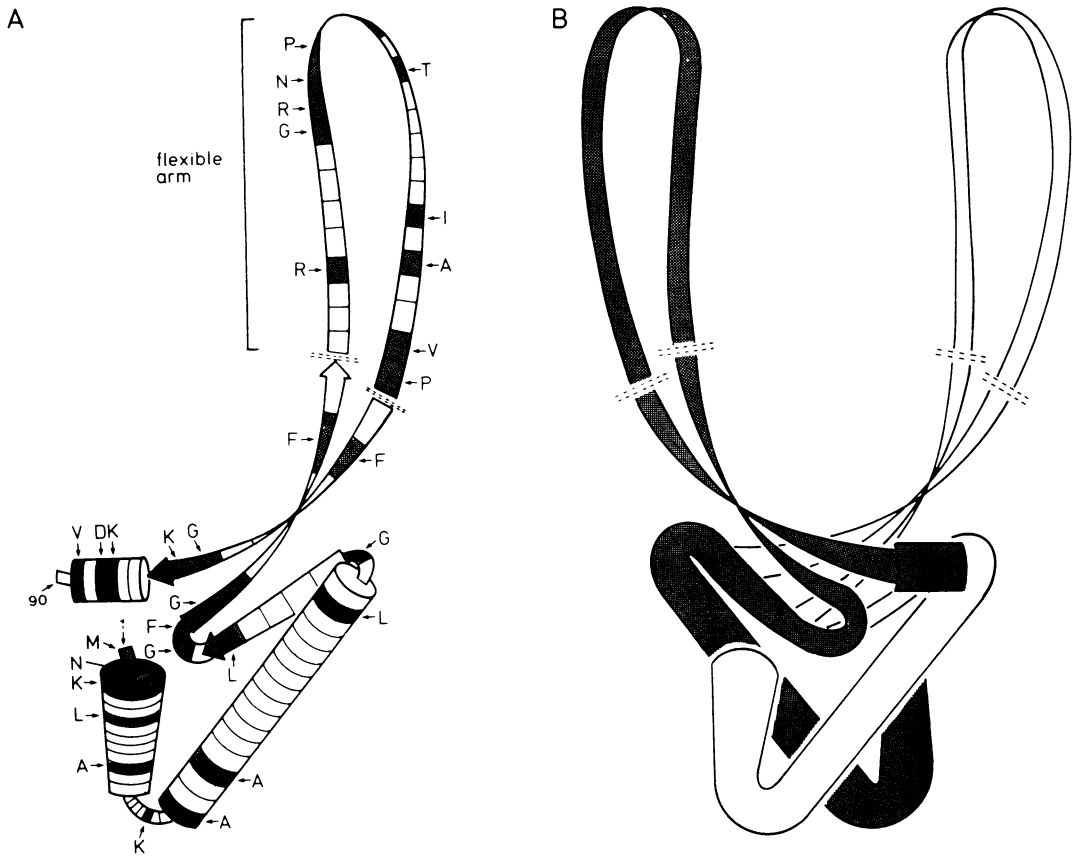

FIG. 2. Structure of HU. The general shape of HU (HBs) has been determined by X-ray crystallography (145). Three regions appear to be alpha helical (residues 3 through 13, 21 through 37, and 84 through 89), and three strands (residues 40 through 45, 48 through 51, and 78 through 83) form an antiparallel beta-pleated sheet. The schematic representations of HU depicted are adaptations of those described by Tanaka et al. (145). It is important to note that the relative positions of the amino acids in these drawings are only approximate. Moreover, the spacial orientation of the amino acids in the regions called flexible arms has not been defined by crystallography; their positions in the figure are arbitrary. (A) HU monomer. The approximate positions of the amino acids are shown; those that are conserved among the proteins listed from the upper six eubacteria in Fig. 1 are shaded. The identity of these conserved amino acids is indicated by the same one-letter nomenclature used in Fig. 1. (B) HU dimer. Two HU monomers interlock to form a dimer. One HU monomer is shaded, while the other is not.

cised when interrelating biochemical studies on *E. coli* HU with structural studies on *Bacillus stearothermophilus* HBs (see below).

HU can also form higher-order structures. Cross-linking studies (HU [129], HD [4], NS [144]), as well as sedimentation measurements (HBs [23]), reveal both trimers and tetramers. With the *E. coli* protein trimers and tetramers tend to be much less stable than dimers, especially at moderate to high salt concentration (106). The *Bacillus* protein, however, tends to be in the tetrameric form (22). As detailed below, in the archaebacterium *T. acidophilum* the tetramer appears to be the basic unit when DNA wrapping occurs. The instability of the HU tetramer from *E. coli* may contribute to the observed instability of *E. coli* chromatin (51).

The *Bacillus* protein HBs has been crystalized, and analysis of X-ray diffraction patterns leads to a model in which two identical monomers interlock (145) (Fig. 2; note that HU from *E. coli* contains nonidentical subunits and thus may differ from the model derived for HBs). The distribution of conserved amino acids suggests that the dimer has a hydrophobic interior (residues 6, 29, 32, 36, 44, 47, 50, and 79; Fig. 1 and 2). The long arms (residues 52 through 77) are flexible and have not been precisely positioned. The bases of the arms and a short region of adjacent amino acids form a concave surface on the HBs dimer which has a diameter of about 2.5 nm; this surface is complementary to the right-handed double helix of DNA.

Three chemical modification studies with HBs support the idea that the long arms bind to DNA. First, DNA-binding ability is lost following photooxidation of the histidine in the arm region (position 54) (76). Second, DNA-binding affinity is lost when arginine residues are modified by 2,3-butanedione (in HBs, four of the five arginines are in the arm region, and the one outside this region appears to react too slowly with 2,3-butanedione to be responsible for loss of DNA-binding ability [76]). Third, interaction of HBs with DNA retards modification of the arginine residues in the arm region (76). Thus an HU dimer can be viewed as a lobster-shaped structure having long arms that bind with DNA. Model-building studies indicate that the arms can encircle DNA so that one dimer of HBs can cover one turn of the DNA double helix, with the arms fitting in either the major or the minor groove (145). The dimer is wedge shaped, and it has been suggested that 8 to 10 dimers could wrap DNA into a nucleosomelike structure having 80 to 100 base pairs (bp)

per turn and a diameter of about 14 nm (145). A similar diameter has been measured by electron microscopy for particles formed by interaction of HU and DNA (126).

Results from crystallographic studies emphasize an important difference between HU-DNA interactions, which have shown little nucleotide sequence specificity, and the site-specific interactions of catabolite gene activator protein, the Cro repressor, and the cI repressor with DNA. HU probably reaches around DNA with its arms, while the latter three proteins appear to dock on one face of the DNA, recognizing symmetric sites by inserting alpha helices into adjacent major grooves (for review, see reference 105).

HU has the ability to compact DNA. Early studies showed that the protein causes the sedimentation coefficient of bacteriophage lambda DNA to increase dramatically (4, 55, 126, 127). Subsequently, electron microscopic studies supported the idea that HU condenses DNA (HD [126, 159]). Wrapping of DNA was demonstrated by the following topological assay. HU was mixed with closed circular relaxed DNA, and the resulting protein-DNA complexes were treated with a topoisomerase to relax superhelical tension arising from wrapping. The DNA was then deproteinized and examined to assess the effect of the topoisomerase. At a protein/DNA weight ratio of 1, HU constrains 14 to 16 negative superhelical turns in simian virus 40 DNA (under similar conditions, eucaryotic histones constrain 21 to 24 negative superhelical turns). After the protein-DNA complexes are treated with glutaraldehyde, beaded structures are observed by electron microscopy; about 14 are generated per simian virus 40 DNA molecule. Thus, under these conditions there are about 275 bp of DNA, 8 to 10 dimers of HU, and a linking change of 1 per bead. It is important to note that the beads are of variable size and that the electron microscopic appearance of the beads depends on how the HU-DNA complexes are prepared for microscopy (A. Misseyanni and J. Rouviere-Yaniv, unpublished observations).

The topological assay described above has also been used to study the cooperativity of HU-DNA interactions (9). No cooperativity was observed when DNA wrapping was measured: linking numbers changed linearly as the HU/DNA ratio increased. Since some earlier studies suggested that binding of HU to DNA is cooperative (60, 91, 127), binding may not always involve wrapping. Supporting this idea is the finding that salt inhibits wrapping well below the concentration required to elute HU from DNA cellulose (126). The *Thermoplasma* protein HTa may behave in a similar way. Binding of HTa to DNA occurs up to salt concentrations of 0.7 M. In contrast, a conformational change in DNA, as assayed by circular dichroism at 282 nm, occurs only below 0.5 M (J. Cook and D. Searcy, unpublished observations). As pointed out in a following section, understanding when HU binding and wrapping of DNA are cooperative will be important in assessing models for chromatin structure.

HU-DNA complexes have been challenged with excess competitor DNA and then assayed for DNA wrapping by the topological method described above (9). At low salt concentration (0.05 M NaCl), DNA wrapping is lost with an apparent half-time of 0.6 min, a value that decreases as the salt concentration increases. If the competitor passively traps HU, these observations suggest that ionic interactions play a significant role in DNA wrapping and that HU may rapidly dissociate from DNA. However, the action of the competitor is not fully understood, for the response of DNA wrapping by HU to competitor DNA does not depend on the superhelical state of the competitor. As noted by Broyles and Pettijohn (9), this is counterintuitive since proteins that unwind DNA or wrap it in a negative sense generally bind more avidly to negatively supercoiled DNA. Whether HU preferentially binds to supercoiled DNA is unresolved. Filter retention of DNA mediated by HU is greater with linear DNA than with supercoiled DNA (57), but HU binds more readily to supercoiled DNA when interacted with DNA in an agarose gel (Misseyanni and Rouviere-Yaniv, unpublished observations).

As with other DNA-binding proteins, HU protects DNA from nuclease digestion (HD [4, 39], NS [31, 110]). But only at high nuclease concentrations and short incubation times can discrete fragments (20 to 150–200 bp) be obtained from HU-DNA complexes (9). The fragments are eventually digested by the nucleases, and the kinetics of digestion are similar to those observed for the loss of DNA wrapping that occurs in the presence of competitor DNA (discussed above [9]). These observations could be explained by a rapid exchange between bound and unbound HU that allows nucleases complete access to the DNA.

HU also creates sites on DNA that are hypersensitive to nucleases (9). Measurements by Broyles and Pettijohn (9) indicate that one series of sites occurs every 58 bp and that another occurs every 8.5 bp. They point out that if the distribution of the nuclease cleavage sites at 8.5-bp intervals reflects the pitch of the helix as it is wrapped around an HU core, then there would be 2 bp fewer per turn when DNA is complexed with HU than when DNA is free in solution or when it is wrapped by eucaryotic histones. A possible consequence of this change is discussed later.

The experiments described above draw us toward the conclusion that HU wraps DNA into nucleosomelike structures. But do bacterial cells contain enough HU to package a major portion of the DNA? Purification studies, which provide minimum estimates for protein abundance, indicate that there are at least 30,000 to 50,000 HU dimers per cell, and the two subunits appear to be present in equimolar amounts as heterodimers (24, 125, 129, 144). When corrected for the amount of DNA in the cell at the growth rate examined, this corresponds to about 20,000 dimers per genome equivalent of DNA or about one dimer per 200 bp of DNA. If in vivo 8 to 10 dimers are required to form one nucleosome having 275 (126) to 290 (9) bp of DNA, then these abundance estimates would allow only one-sixth of the genome to be packaged into nucleosomelike particles. Thus the possibility of locally high concentrations of HU on DNA must be considered. A cytological study supports this idea, suggesting that HU may be concentrated around the periphery of the nucleoid (6).

Integration Host Factor

Several site-specific recombination systems in *E. coli* are strongly stimulated by a host factor called IHF (integration host factor). Nash and his collaborators have developed methods for purifying IHF, using as an assay the ability of the protein to stimulate Int-mediated recombination of bacteriophage lambda (100). Two heat-stable polypeptides, in approximately equimolar amounts, copurify with IHF activity. The genes encoding the two proteins (*himA* and *hip*) have been cloned and sequenced, and the molecular weights of the proteins have been calculated to be 11,224 for IHF-α (89, 92) and 10,581 for IHF-β (33). Recently, strains have been constructed that simultaneously overproduce the alpha and beta subunits of IHF, greatly simplifying the production of large amounts of highly purified IHF (H. Nash, E. Flamm,

TABLE 3. Amino acid sequence homologies among HU-like proteins

Protein	% Identical amino acids[a]										
	HU-β	HU-α	HPa	HBs	HRm	HCp	HAn	IHF-α	IHF-β	HTa	TF1
HU-β	—										
HU-α	69	—									
HPa	87*	66*	—								
HBs	58	59	63*	—							
HRm	52	49	58*	59	—						
HCp	53	49	57*	61	47	—					
HAn	43	47	46*	54	46	58	—				
IHF-α	32	37	31*	42	41	40	34	—			
IHF-β	34	33	39*	39	34	38	33	32	—		
HTa	27	27	28*	32	28	33	33	27	25	—	
TF1	34	39	39*	47	29	33	32	26	25	23	—

[a] Determined in two-by-two comparisons of data presented in Fig. 1. The percentages are based on the 90 numbered amino acids in Fig. 1, except in the case of HPa, for which only part of the sequence (67 amino acids) is available. Since comparisons including HPa may show artificially high values, they are marked with asterisks. Species abbreviations are the same as listed in the legend to Fig. 1.

R. Weisberg, and H. Miller, personal communication). As with HU, IHF has a native molecular weight of slightly more than 20,000 (100). Thus, it has been attractive to think of IHF as a heterotypic dimer, although this feature has not been clearly demonstrated.

IHF was first shown to be a DNA-binding protein by its ability to cause DNA to be retained by a membrane filter (100). Protection studies then identified three sites on *attP*-containing DNA where IHF binds (15). The sites are 30 to 40 bp long, and they share a common recognition sequence. IHF recognition sites are also found in the *att* region of bacteriophages φ80 and P22 (82), the termini of the insertion element IS1 (P. Gamas, M. Chandler, P. Prentki, and D. Galas, personal communication), in the phage 21 *cos* site (32), and upstream from translation initiation codons or close to promoters of several genes whose expression is influenced by mutations in the genes encoding IHF (15, 34, 70). The sequences for nine binding sites have been determined (15, 82; Games et al., personal communication), and the following consensus sequence has been derived: Py-A-A-X-X-X-X-T-T-G-A-T-(A or T).

The many amino acid sequence homologies between IHF and HU (Fig. 1; Table 3) and the tendency for HU to copurify with IHF and vice versa (100) suggest that the two might share common features. An involvement in wrapping DNA appears to be one. Statements about the participation of IHF in DNA wrapping are derived from experiments in which IHF and Int bind to an *attP* recombination site, and they are discussed below in the context of IHF function. An important contrast between IHF and HU is their relative abundance: many copies of HU are present in cells (24, 125), while the concentration of IHF is thought to be low (97).

Protein H

Protein H was first recognized through its activity as an inhibitor of DNA synthesis in vitro (59). The protein has a molecular weight of 28,000 when measured by gel electrophoresis in the presence of the detergent sodium dodecyl sulfate, and its apparent molecular weight is about 56,000 when measured by gel filtration. Thus, as with HU, H is probably a dimer in dilute solution. Its amino acid composition is similar to that of eucaryotic histone H2A, and its inhibitory activity is stable after treatment at pH 2 or at high temperature.

H is a DNA-binding protein, interacting with both double-stranded and single-stranded DNA up to a salt concentration of 0.1 M. This maximal salt concentration is low compared with that observed for HU-DNA binding, suggesting that H may not bind DNA as strongly. The protein facilitates reassociation of denatured DNA, a property blocked by antiserum raised against eucaryotic histone H2A. Maximal DNA reassociation activity occurs at a protein/DNA ratio that corresponds to 1 dimer of H per 75 nucleotides of DNA, a ratio similar to that found for the inhibitory aspects of protein H activity. The protein abundance is reported to be at least 120,000 monomers per cell (84). Little else has been reported about this protein, and it is not known if it participates in packaging DNA.

Protein H1

H1 is another small, moderately abundant DNA-binding protein found in bacteria (18). Unlike HU and H, H1 is a neutral rather than a basic protein. Thus, on the basis of its amino acid composition, it would not be classified as a histonelike protein (75). However, the protein binds very strongly to DNA (75) and may be able to compact it (140).

In solution H1 appears to be a dimer, although small amounts of trimers and tetramers have also been detected (139). Recent studies show that preparations of protein H1 contain three polypeptides, each having a molecular weight of 15,500 (140). The three species, called H1a, H1b, and H1c, differ in isoelectric point. Purification studies indicate that together they add up to about 15,000 copies per cell in roughly equimolar amounts when assayed in exponentially growing bacteria (140). When cells pass into stationary phase, species H1a increases in abundance until it is more than four times as abundant as each of the others, and together there are about 26,000 copies of the three H1 proteins per cell (140).

H1 binds strongly to DNA; 0.5 M salt is required to elute the protein from DNA cellulose (75). Protein cross-linking carried out after H1 is bound to DNA indicates that about 50% of the protein is in a dimeric form and 25% is in a tetrameric form (139). There is no evidence for cooperative binding or clustering of the protein complexes on DNA, and saturation appears to occur when H1 would cover <8% of the DNA molecule being tested (139). Under these conditions ethidium bromide binding decreases, and at low salt concentration a combination of H1 and ethidium bromide causes DNA to precipitate.

Once H1 was fractionated into three species, DNA-binding studies focused on the most abundant one, H1a

(140). H1a shows no binding preference for supercoiled DNA relative to relaxed DNA. Moreover, the protein exhibits little effect on DNA topology: the relaxing effect of topoisomerase I is only slightly altered. Thus there is no evidence that H1a by itself wraps DNA in a way similar to that observed with HU. Nevertheless, complexes between H1a and DNA do sediment more rapidly than expected simply from the increase in mass, and they migrate as discrete bands during gel electrophoresis. Whether these complexes arise from DNA compaction or aggregation has not been determined.

FirA

Mutations in *firA* reduce or eliminate rifampin resistance in *E. coli* conferred by (Rif^r) *rpoB* mutations (*rpoB* encodes the beta subunit of RNA polymerase). Little is known about the *firA* gene product. A temperature-sensitive *firA* mutant lacks a 17,000-dalton protein (79), suggesting that this protein is the product of either the *firA* gene or a gene controlled by *firA*. The protein is heat stable and acid soluble, binds to DNA, and is present at about 20,000 copies per cell (79, 81).

HISTONELIKE PROTEINS FROM ARCHAEBACTERIA

The most thoroughly studied histonelike protein from archaebacteria is HTa from *T. acidophilum* (for review, see reference 134). This organism is an extreme thermoacidophile originally isolated from a burning tailings pile of low-grade coal refuse. The optimal culture conditions for the organism are 59°C at a pH of 1 to 2. The extreme growth conditions suggested that the organism might contain proteins specialized in stabilizing DNA. Nucleoprotein was isolated from the cells, and when it was purified by gel filtration chromatography, DNA was found to be complexed to a single abundant protein (132, 136). The protein, called HTa, was purified and characterized as described below.

HTa is a small, basic DNA-binding protein. Its molecular weight, determined from the amino acid sequence, is 9,934 (21), and 23% of the residues are basic amino acids (Fig. 1). In solution the protein behaves as a tetramer during gel exclusion chromatography (136). The protein binds avidly to double-stranded DNA cellulose, eluting at sodium ion concentrations between 0.7 and 0.8 M (20). This salt concentration is much higher than the intracellular concentration of 0.05 M (133), indicating that binding to DNA is probably quite strong in vivo. As with many other DNA-binding proteins, HTa stabilizes DNA against thermal denaturation. The stabilizing effect can be as high as 40°C (134, 142), and it has been suggested that one of the roles of the protein is to protect chromosomal DNA from thermal denaturation. Part of the thermal stability of the protein itself may arise from the presence of five phenylalanine residues that appear to be buried in the hydrophobic core of the protein (134).

The nucleoprotein complex isolated from *Thermoplasma* cells has been characterized in several ways. The ratio of HTa/DNA (by weight) is about 0.4 (136), and treatment of isolated nucleoprotein with micrococcal nuclease indicates that about 15 to 25% of the DNA is protected by the protein from digestion. This corresponds to about 40 bp of DNA per tetramer of HTa (136). From cross-linking studies tetramers of HTa are known to exist in the nucleoprotein preparations. Electron microscopic examination of nucleoprotein reveals that globular structures (5 nm in diameter [102, 136]) occur along the DNA such that the contour length of the DNA is reduced by an amount equivalent to 40 bp per globular unit (136). The spacing of these nucleosomelike particles does not appear to be uniform because nuclease digestion fails to generate the distinct oligomeric series of DNA fragments characteristic of eucaryotic chromatin.

Nucleoprotein has also been isolated from three other archaebacteria. Two proteins are found in nucleoprotein from *Sulfolobus acidocaldarius* (49), a basic one with a molecular weight of 14,500 (HSa) and a nonbasic one having a molecular weight of 36,000. The smaller protein is probably a dimer in dilute solution. A small fraction (5%) of the nucleoprotein complex is resistant to digestion by micrococcal nuclease. As with HTa from *T. acidophilum*, higher temperatures are required to denature DNA when complexed to HSa than when the DNA is free in solution, but the effect with the protein from *S. acidocaldarius* is not as dramatic as with that from *T. acidophilum*. A different purification procedure produces two proteins from *S. acidocaldarius* that are smaller than those described above (146). Relationships among these proteins have not been defined. Two small proteins have also been purified from another species, *S. solfataricus* (146). The amino acid sequence of the smaller one has been determined, and it appears to be unrelated to the HU proteins (67). Five small proteins are associated with the DNA of *S. brierlevi* (146). Another archaebacterium studied, *Methanosarcina barkeri*, appears to have only one major nucleoprotein, a basic protein having a molecular weight of 14,500 (13). A similar finding was made for *Methanobacterium thermoautotrophicum* (146).

PHYLOGENETIC RELATIONSHIPS

Early in the study of histonelike proteins it was discovered that antibodies raised against HU from *E. coli* cross-react with a histonelike protein isolated from a cyanobacterium (55), suggesting that HU is an evolutionarily conserved protein. Subsequent studies showed that cross-reactivity occurs with histonelike proteins from a number of eubacteria (146) and that it even extends to a protein from plant chloroplasts (8). Functional homologies have also been reported: HAn (*Anabaena* sp.) will substitute for HU (*E. coli*) in both initiation of DNA replication (24) and transposition (16).

Amino acid sequence analyses are now complete for a number of the HU-like proteins. Homologies are easily seen (Fig. 1 and 2), particularly in hydrophobic regions and in the DNA-binding arms. Table 3 lists two-by-two comparisons of the sequenced proteins, showing that approximately half of the residues are identical among proteins from eubacteria. Not included in Fig. 1 and Table 3 are sequence analyses for the HU analog from *Synechocystis* sp., a cyanobacterium; 40% of the residues determined are identical to those in the *E. coli* protein (1). The level of homology drops to about one-fourth of the residues when eubacterial proteins are compared with the archaebacterial protein HTa (Table 3). As pointed out by Flamm and Weisberg (33), extensive homologies exist among the HU proteins and the two subunits of IHF. The two proteins of IHF are slightly more related to the HU proteins than is HTa. Surprisingly, the two proteins of IHF are no more related to each other than they are to the HU proteins when amino acid identities are considered. A common ancestor may have given rise to three descendents: IHF-α, IHF-β, and a primitive HU. Much later the primitive HU may have diverged to give rise to HU-α and HU-β which are now almost 70% homologous. The genes encoding the

HU subunits, *hupA* and *hupB*, are not as highly conserved in their nucleotide sequences as in their amino acid sequences (F. Imamoto, personal communication).

In some organisms (*E. coli* [73], *Synechocystis* sp. [1], and *Salmonella typhimurium* [D. Prigent and J. Rouviere-Yaniv, unpublished observations]) HU proteins exhibit sequence heterogeneity, indicating that the cells contain at least two species of the protein and that the protein probably exists as a heterotypic dimer (129). In others there appears to be only one type of HU protein (*Rhizobium meliloti* [71, 72], *Pseudomonas aeruginosa* [56], and *B. stearothermophilus* [68]). The physiological and phylogenetic significance of this observation is not yet known.

Amino acid analyses also suggest that the HTa protein from *T. acidophilum* may be intermediate between eucaryotic histones and eubacterial HU-like proteins. If the N terminus of HTa is properly aligned with the sequence of histone H2A, there is 25% homology in the first 24 amino acids (140). As pointed out above, HTa is about 25% homologous with the bacterial proteins. But there is little or no sequence homology between the eubacterial HU proteins and the eucaryotic histones (73, 135).

Analyses of nuclei from dinoflagellates, a diverse group of lower eucaryotes, suggests that these organisms may reflect an important step in evolution. One group of dinoflagellates is uninucleate; in these species the chromosomes are permanently condensed (25) and appear to lack histones (122). Analysis of chromatin preparations from one of these species, *Gyrodinium* (*Crypthecodinium*) *cohnii*, reveals the presence of a single, small (13,000-dalton), acid-soluble protein having a high lysine content (118, 121, 122). Morphologically, the chromatin fibers from *G. cohnii* appear smooth, lacking the beaded, nucleosome structure characteristic of higher organisms (54, 119). Another group of dinoflagellate contains two dissimilar nuclei within a single cell. One nucleus is morphologically similar to nuclei observed in uninucleate dinoflagellates and has permanently condensed chromosomes. This nucleus has been termed the dinocaryotic nucleus (120). The other nucleus, called a eucaryotic nucleus, exhibits no distinct chromosomes. When the eucaryotic nuclei are isolated, they are found to contain four basic proteins that coelectrophorese with four of the five calf thymus histones (120). In addition, chromatin isolated from eucaryotic nuclei has the subunit structure characteristic of chromatin from higher organisms (119). Thus the intriguing possibility exists that the binucleate dinoflagellate arose from the fusion of a primitive eucaryotic alga with a uninucleate dinoflagellate (117).

Organelles from higher cells lack histones, but, like the uninucleate dinoflagellates, they contain a histonelike protein. The histonelike protein from spinach chloroplasts cosediments with the chloroplast nucleoid, has a molecular weight of 17,000, and cross-reacts with antisera against HU (8). The cross-reaction of the chloroplast protein is stronger with HU from *Synechocystis* sp. than with HU from *E. coli*, suggesting a closer relationship with cyanobacteria; relationships with the dinoflagellate protein have not been reported. The yeast mitochondrial protein (12) has a molecular weight of 20,000. It does not cross-react with antisera against HU, but it does contribute to the introduction of supercoils into DNA if protein-DNA mixtures are treated with a eucaryotic chomatin extract containing nicking-closing activity. It appears that the mitochondrial protein is encoded by a nuclear gene, and it may be that chloroplasts and mitochondria have independent origins.

Even bacteriophages encode histonelike proteins. The best-studied example is TF1, a protein isolated from *B. subtilis* after infection with bacteriophage SPO1 (50). This protein is related to HU (Fig. 1), and it cross-reacts with serum raised against HU from *E. coli* (E. P. Geiduschek and J. Rouviere-Yaniv, unpublished observations). SPO1 DNA contains hydroxymethylcytosine, and it may be that the presence of the base modification makes it necessary for this virulent bacteriophage to encode its own form of HU. The gene encoding TF1 has been cloned and sequenced (50), so it should be possible to obtain mutations that will lead to a better understanding of the function of TF1 and, we hope, HU.

The HU-like proteins appear to be excellent markers for examining phylogenetic relationships. They occur in organisms that are only distantly related (*E. coli* and cyanobacteria are thought to have diverged 2×10^9 to 3×10^9 years ago), and their rate of evolution is slow (about 1% difference in amino acid sequence per 5×10^7 years, a rate comparable to that estimated for eucaryotic histones H2A and H2B) (1).

BACTERIAL CHROMATIN STRUCTURE

HU was discovered at a time when our understanding of eucaryotic chromatin structure was advancing rapidly. A number of similarities between HU and histones soon emerged: both are small, basic, DNA-binding proteins. Moreover, both are abundant and both are evolutionarily conserved. Subsequent cell fractionation studies have supported the notion that bacterial DNA is packaged into a chromatinlike structure containing HU, but so far this type of study has not produced a clear definition of bacterial chromatin. Below we outline observations that bear on bacterial chromatin structure.

DNA Topology

Wrapping of DNA around histones to form nucleosomes is one of the key features of eucaryotic chromatin (for review, see reference 88), and it is likely that DNA wrapping is also important in bacterial DNA packaging. Since DNA topology can be an important parameter when considering DNA wrapping, we begin the consideration of chromatin structure by briefly discussing DNA topology and supercoiling. By current conventions, wrapping DNA into a left-handed toroidal coil is topologically equivalent to DNA writhe having a negative sense. One consequence of such a wrapping is that removal of the proteins involved in wrapping would leave the DNA under negative superhelical tension, an energetically activated condition that can influence the binding of proteins to DNA (for discussion, see reference 41). Negative supercoils can also arise from the action of a topoisomerase. Since a number of chromosomal processes, such as replication, transcription, and recombination, are affected by supercoiling or topoisomerase activity or both (for review, see reference 6), it is likely that DNA supercoiling is an important aspect of chromatin structure.

Several lines of evidence support the idea that DNA in bacterial cells is under negative superhelical tension. The most direct evidence comes from psoralen-binding studies. These are based on the observation that purified DNA binds more psoralen when supercoiled than when nicked. In living bacterial cells intact DNA also binds more psoralen than DNA relaxed by gamma irradiation (137). Thus, at least part of the DNA must be under superhelical tension. In contrast, no superhelical tension is detected by this assay in eucary-

otic cells, emphasizing that the majority of the chromatin in higher cells differs from chromatin in bacteria.

Another indication that intracellular DNA is under superhelical tension is obtained from topological measurements following Int-mediated recombination (6a). In this system the complexity of catenated products formed in vitro from intramolecular recombination is proportional to the superhelix density of the substrate DNA. Examination of the complexity of the catenated products formed by recombination in vivo has led to the conclusion that plectonemic supercoils are present in vivo and that they account for about 40% of the superhelical density measured in deproteinized DNA (6a).

Physiological perturbations of topoisomerase activities also support the idea that superhelical tension is an important parameter in bacterial DNA structure. Bacterial cells contain both relaxing and supercoiling activities. In vitro, topoisomerase I (152), gyrase (44), and topoisomerase III (19) can remove negative supercoils from DNA, and gyrase can introduce negative supercoils (for reviews of topoisomerase biochemistry, see references 14, 41, and 153). The availability of inhibitors and mutations has made it possible to examine topoisomerase activities in vivo by measuring titratable supercoiling in DNA isolated from the treated cells. For example, inhibitors of gyrase block the introduction of titratable supercoils into bacteriophage lambda during superinfection of a lysogen (43, 45) and lead to a loss of supercoils from the bacterial chromosome (28, 86). Mutations in *topA*, the gene encoding topoisomerase I (143, 147), can lead to higher-than-normal levels of titratable supercoiling in both the chromosome and plasmids (112, 113, 115). Thus it appears that both gyrase and topoisomerase I participate in controlling supercoiling. No mutations or inhibitors are available to block topoisomerase III activity; consequently, little can be said about the function of this enzyme. Although the studies cited above involve supercoiling measurements made after extraction of DNA, inhibition of gyrase by coumermycin causes the same amount of relaxation as gamma irradiation when measured in vivo by the psoralen-binding method (137). Thus it is likely that topoisomerase activities directly affect the level of intracellular DNA superhelical tension.

Measurement of supercoiling also contributes to one of the lines of support for the existence of a chromatinlike structure in bacteria. Pettijohn and Pfenninger (109) postulated that if proteins wrap a significant fraction of the DNA into nucleosomelike structures and constrain supercoils, then treatments that relax superhelical tension without perturbing the wrapping should fail to remove all of the supercoils in the DNA. Indeed, inhibition of intracellular gyrase, using drugs or temperature-sensitive mutations, fails to completely eliminate supercoiling when measured in isolated DNA (28, 45, 141). So does gamma irradiation (109). In the latter case over 95% of the plasmids inside cells were nicked, and after a brief incubation to allow DNA repair under conditions in which gyrase was inhibited, plasmid DNA was isolated and supercoiling was measured. Only half of the supercoils had been relaxed by the nicking treatment, as if the other half were constrained into some type of chromatin structure. These numbers fit well with the estimate by Bliska and Cozzarelli (6a) that the level of intracellular superhelical tension is 40% of that found if the DNA is deproteinized.

Chromatin Morphology

Using electron microscopy, Griffith (51) examined very gently lysed *E. coli* cells and observed 12-nm filaments having an axial repeat of about 13 nm. These structures are reminiscent of eucaryotic nucleosomes. Cells containing circular bacteriophage lambda DNA were examined, and contour lengths of naked DNA and "chromatin" were determined. From these data Griffith calculated that the packing ratio is between six- and seven-fold, close to the packing ratio for eucaryotic nucleosomes. According to these calculations, if each nucleosomelike particle has a diameter of 13 nm, then each would contain between 220 and 290 bp of DNA (assuming 0.34 nm per base pair). Similar structures were found with linear DNA, suggesting that a topologically closed system is not required to maintain chromatin structure in bacteria. Although the nucleosomelike particles described by Griffith have been observed by others (63, 87), these structures are very labile and have not been isolated and characterized biochemically.

Cell Fractionation Studies of Chromatin

When bacterial nucleoids are isolated using low salt concentrations, HU is found associated with chromosomal DNA (125, 150). The idea that HU is bound to DNA in vivo is supported by the observations that the majority of the cellular HU is bound to the chromosome whereas HU added exogenously to cell lysates does not bind avidly to nucleoids (125). By partially digesting chromosomal DNA with endogenous nucleases, Varshavsky and colleagues were able to obtain DNA fragments bound to protein (149, 150). In their studies two species comprised the bulk of the DNA-bound protein, one of which was identified as HU (150). The other protein has not been clearly identified. It may have been FirA, H1, or a still unidentified protein. Varshavsky et al. (150) cross-linked the proteins to DNA, and from density determinations they estimated that, on average, there is one monomer of each protein per 150 to 200 bp of DNA. Nuclease digestion did not release large fragments of naked DNA, so it appears that there are no large, protein-free stretches of DNA in these preparations. A digestion pause at 120 bp of DNA was observed during treatment of the chromatin with micrococcal nuclease. Attempts to find the type of repeating array of particles characteristic of eucaryotic chromatin have not been successful (125, 150).

In another approach, an insoluble, viscous nucleoprotein complex was isolated which contained about 30 proteins (138). Digestion with micrococcal nuclease solubilizes the DNA component of the complex, and after limited digestion it is possible to obtain material sedimenting at 10S to 11S. The 10S to 11S structures contain a subset of the proteins associated with the nucleoprotein complex; HU was not one of the proteins enriched in the 10S to 11S material.

A third approach has focused on isolating protein-DNA complexes from plasmids. Buc and his collaborators have initiated this type of study by partially purifying plasmid-protein complexes by gel filtration. The plasmid-containing complexes are stable enough to be sedimented into sucrose density gradients where they sediment almost twice as fast as purified supercoiled DNA (10, 155). There are about 10 proteins associated with the complexes. The dominant one appears to be H1, which is present at a stoichiometry of about one monomer per 90 bp (10, 140, 155). HU is present at about one monomer per 250 bp of DNA (155). These plasmid studies also indicate that the topology of the DNA is not crucial for maintaining the structure of the complex since the protein composition is not altered by cleaving the DNA with a restriction endonuclease (10). Data presented by Imamoto's group (157) confirm the association of HU with plasmid DNA.

Perspective on Bacterial Chromatin

Topological and electron microscopic investigations indicate that bacterial DNA is organized into a wrapped chromatinlike structure, but so far the cell fractionation approach has failed to provide a clear biochemical definition for bacterial chromatin. Five small proteins, HU, IHF, H, H1, and FirA, have been loosely categorized as histonelike. HU has the characteristics expected of a protein that condenses large regions of DNA. It is abundant, wraps DNA with little sequence specificity, and is associated with both chromosomal and plasmid DNA. At a 1:1 HU/DNA weight ratio, purified HU introduces a linking change of −1 for every 270 to 290 bp of DNA (9, 126), a numerical relationship that is close to the estimate of one nucleosomelike particle per 220 to 290 bp found in DNA from gently lysed cells (51).

IHF is structurally related to HU, and it can also wrap DNA in vitro. This protein, in conjunction with Int, appears to wrap *attP*-containing DNA (for discussion, see next section). Alone, IHF can introduce one negative topological turn when a 600-bp fragment containing *attP* is ligated to form a circle (H. Nash, personal communication). This wrapping phenomenon is not observed in the presence of Int (52); consequently, it may reflect a bacterial function rather than being an essential feature of IHF action during phage integration and excision. Since IHF binding to DNA is sequence specific, IHF is not abundant (97), and the protein has not been identified as a component of putative chromatin obtained by cell fractionation, it is unlikely that IHF participates in a general way in DNA packaging.

Cell fractionation studies suggest that H1 is associated with cellular DNA (10, 140, 150, 155), but that DNA was not shown to be wrapped into nucleosomelike structures. Nor is H1 known to wrap DNA in vitro. The other two proteins, H and FirA, have not been recovered associated with putative chromatin. Additional information about these three proteins is required before they can be assigned a role in chromatin structure.

A number of details have been described concerning the interaction of HU and DNA. In Fig. 3 we have sketched a model which fits many of the numerical estimates obtained from these studies. In this model 290 bp of DNA is wrapped around five adjacent tetramers of HU. The model depicted in Fig. 3 differs radically from the customary nucleosomelike models in which the DNA makes a single turn around 8 to 10 HU dimers (for example, see reference 145), and it is important to emphasize that the model in Fig. 3 relies heavily on the interpretation by Broyles and Pettijohn (9) that the nuclease-sensitive sites occuring at 8.5-bp intervals reflect the pitch of the DNA (for details, see the legend to Fig. 3). An important distinction between the two types of model is that the one shown in Fig. 3 is symmetrically open; i.e., the structure would be fundamentally unchanged if the number of adjacent HU tetramers were higher or lower than five. Thus one would expect additional factors, such as specific nucleotide sequences or other proteins, to be involved in the interaction if the complex always contains five tetramers of HU. If long runs of adjacent HU tetramers are associated, the model would readily accommodate the cooperative binding of HU to DNA observed when measuring the effect of HU on DNA melting temperature (NS [91]) and on retention of DNA by membrane filters (HBg [60, 127]). Cooperative binding also fits with the electron microscopic observation that rod-shaped structures of variable length form when HU and DNA are mixed (60). One prediction of the model is that cleavage products following limited nuclease digestion will have lengths that are integrals of 58 bp. Attempts to demonstrate a series of DNA fragments have not been successful (136, 150).

A model in which many HU tetramers can associate side by side, such as that shown in Fig. 3, does not explain how linking changes can correspond to the number of nucleosomelike particles observed by electron microscopy (126). Perhaps in vitro a variety of structures can form, and small differences in the reaction conditions determine which type predominates. A variety of structures can be seen by electron microscopy when HU is interacted with supercoiled DNA (Fig. 4). Some of the structures appear to contain variable-length loops of DNA, a feature not easily explained by either nucleosomal or side-by-side models. The loops may represent intermediates in the formation of more condensed structures, and the variable size of the loop may account for the variable size of the beadlike structures reported previously (126).

A separate consideration is whether bacterial chromatin is a dynamic or a static structure. Two reconstitution studies suggest that it may be dynamic. In both cases excess competitor DNA added after reaction of HU and DNA led to detection of rapid dissociation of HU from DNA (9, 60).

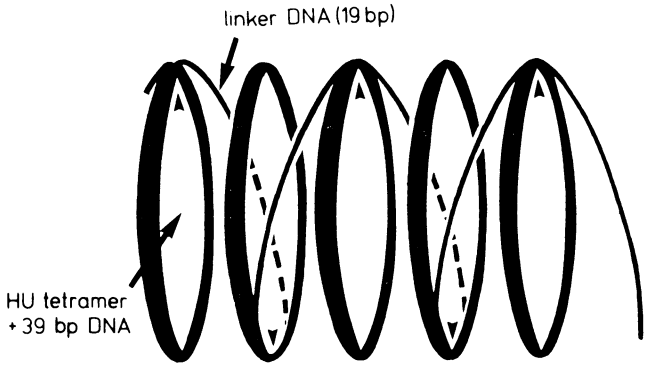

FIG. 3. Model for DNA wrapping by HU. DNA is wrapped around tetramers of HU such that 290 bp makes 7.5 turns around five tetramers of HU. Each turn consists of 38 to 39 bp, and each linker is about 19 bp long. Thus adjacent tetramers are inverted relative to each other. The model is based on the following numerical considerations. For each linking change of −1, HU binds to about 290 bp of DNA (9, 126). Sites hypersensitive to nucleases occur at 58-bp intervals (9), so some structure occurs five times for each change in link of 1 (290/58 = 5). The repeating structure is probably a tetramer of HU since 20 monomers bind per 290 bp of DNA (90, 126). Nuclease-sensitive sites also occur every 8.5 bp (9). If this reflects a change in the helical pitch of DNA to 8.5 bp per turn, there would be 2 bp fewer per turn than found with DNA in solution. Over the 290 bp of wrapped DNA, there would be 6.5 turns of additional twist (DNA in solution has a twist of about 10.5 bp per turn or 34.28° per bp; if the twist is changed to 8.5 bp per turn or 42.35° per bp, then the difference is 8.1° per bp, 2,349° per 290 bp, or 6.5 turns). According to the topological relationship $W = L - T$, where W is writhe, L is linking number, and T is twist, for each 290 bp of DNA complexed with HU, writhe should change by −7.5 (L = −1; T = +6.5). This would mean that, if a nucleosomelike particle has a linking change of −1, as suggested by comparison of linking changes with particles observed by electron microscopy (126), then the DNA would describe a path having 7.5 helical turns (1.5 turns per tetramer of HU). Each turn would have about 39 bp of DNA (about 39 bp are associated with each tetramer of the *Thermoplasma* protein HTa [136]). Assignment of 19 bp to the linker region might generate the hypersensitive sites found at 58-bp intervals (39 + 19 = 58).

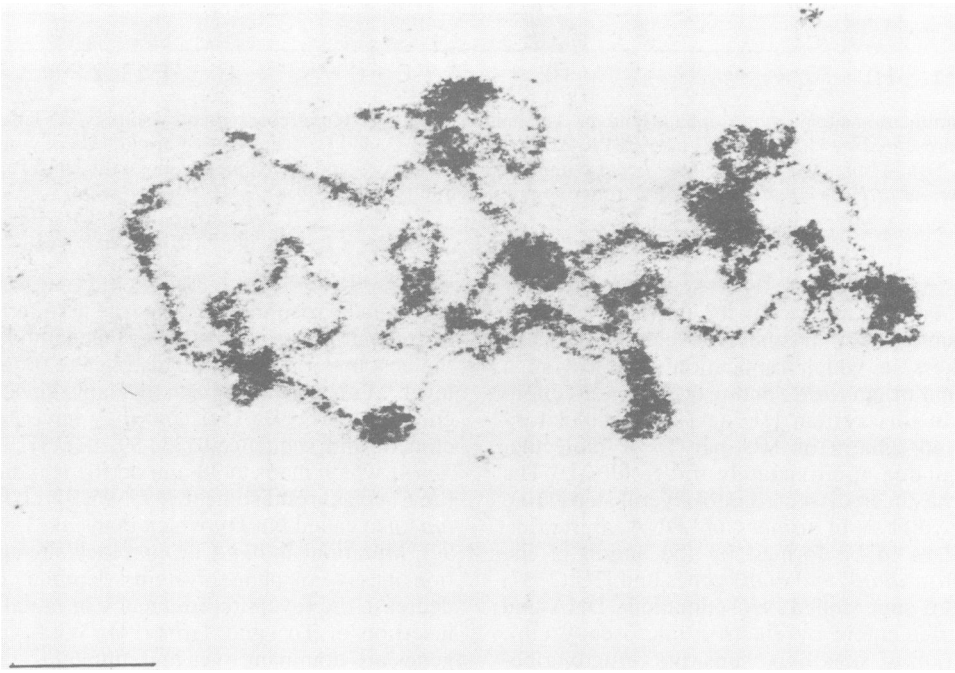

FIG. 4. HU bound to supercoiled DNA. HU was complexed with supercoiled simian virus 40 DNA at a weight ratio of 0.5, and the complexes were adsorbed to activated carbon-coated grids. Samples were then stained briefly with uranyl acetate and visualized by transmission electron microscopy. Bar, 0.5 μm.

Whether the dissociation occurs either in the absence of competitor DNA or with isolated chromatin is not known.

FUNCTIONS OF HISTONELIKE PROTEINS

HU

No mutant phenotypes have been reported for HU; consequently, we have no physiological support for specific functions. However, progress is being made with genetic analysis of HU. The gene *hupB*, which encodes HU-β (HU1, NS1), has recently been cloned, sequenced, and mapped (62, 62A). It is located between *proC* and *minA* at 10 min on the standard genetic map of *E. coli* K-12. Fine mapping shows that *hupB* begins near the 3′ end of *lon* and that both *lon* and *hupB* are transcribed in a clockwise direction. Recently, *hupA* has also been cloned (Imamoto, personal communication).

Several biochemical tests have been performed with HU that suggest functions. One potential function of HU involves transcription. When the isolation of HU was originally reported, the protein was found to stimulate transcription from a bacteriophage lambda DNA template by approximately sixfold at an HU/DNA weight ratio of about 1 (127). In this assay HU appears to affect the template since optimal stimulation requires more HU protein when the amount of template is increased but RNA polymerase concentration is kept constant. In another case HU (HD) showed a much lower level of stimulation (4), and in subsequent studies HU (HPa, NS) was reported to inhibit transcription (56, 83). The differences among the various laboratories have not been resolved; it is difficult to assess the effects impurities such as IHF might have on transcription assays. Also not addressed are differences arising from isolation procedures. HU in its native form is 90% αβ heterodimer (129), and this structure could differ in activity from NS1 and NS2 prepared in 6 M urea (144). An involvement of HU in transcription could explain why cytological observations place the protein around the periphery of the nucleoid (6) where nascent RNA tends to be localized (131). At the molecular level an attractive speculation is that the DNA wrapping activity of HU promotes DNA loop formation between distant nucleotide sequence elements known to influence transcription. It has been proposed that loop formation is a way to bring together separated binding sites of regulatory proteins for more effective gene control (for review, see reference 114).

HU is also involved in three types of site-specific recombination in which proper orientation and positioning of nucleotide sequences are important. One concerns transposition by bacteriophage Mu. Only three proteins, MuA, MuB, and HU, are required for the initial strand transfer reaction that generates a transposition intermediate (16). The second case involves transposition by Tn*10*. HU, as well as IHF, stimulates an in vitro transposition assay (D. Morisato and N. Kleckner, personal communication). The third case is the site-specific inversion associated with flagellar phase variation in *Salmonella* spp. This reaction appears to require two proteins, the *hin* recombinase and a protein called factor II. The reaction is stimulated about 10-fold by HU (60a). The optimal effect of HU occurs when about 40 to 50 dimers of HU per substrate DNA molecule are present in the reaction. The wild-type system includes an enhancer sequence to which factor II binds, and the location of the enhancer is important for the stimulatory effect of HU: placement of the enhancer far from the recombination sites lowers the stimulatory effect of HU.

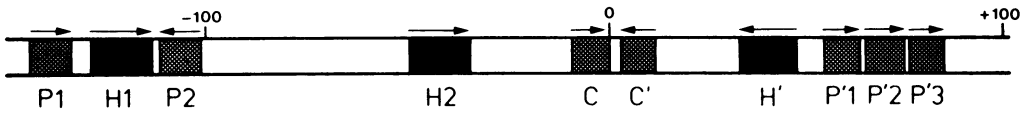

FIG. 5. *attP* recombination site of bacteriophage lambda. The binding sites of IHF are represented by solid boxes labeled H1, H2, and H'. Those for Int are represented by shaded boxes labeled P1, P2, P'1, P'2, P'3, C, and C'. The relative orientation of consensus sequences is indicated by arrows. Strand breakage and exchange occur within the core regions C and C'. Numbers above the DNA represent the standard nucleotide coordinates of *attP*. The figure is adapted from one presented in reference 16.

HU also affects in vitro assays for the initiation of DNA replication. A plasmid system has been developed by Kornberg and co-workers in which replication begins from a cloned chromosomal origin (*oriC*) and proceeds bidirectionally from it (61). In this system HU acts as a stimulatory factor. At about 40 dimers of HU per DNA molecule, replication is stimulated approximately threefold (24). The initiation reaction has been dissected into several steps (103, 148). Prior to the synthesis of primers and DNA, a prepriming complex appears to be formed by the action of the products of the *dnaA*, *dnaB*, and *dnaC* genes and HU (2, 37, 148). DnaA binds to supercoiled *oriC*-containing DNA and forms a complex detectable by electron microscopy (36). From the distribution of sites hypersensitive to deoxyribonuclease I, it appears that DNA is wrapped around DnaA (36). By combining immunological and electron microscopic techniques, several additional statements can now be made about the prepriming complex (B. E. Funnell, T. A. Baker, and A. Kornberg, unpublished observations). From the size of the complex it has been estimated that 20 to 40 monomers of DnaA are present. Addition of DnaC, DnaB, and HU enlarges the complex; DnaA, DnaB, and HU are present in the larger complex, which appears to include an additional 50 bp of DNA from the left side of *oriC*. After formation of the prepriming complex, addition of single-strand binding protein then allows the helicase activity of DnaB to open a small bubble at *oriC*, and this bubble is enlarged if gyrase is added to relieve torsional tension (2; Funnell et al., unpublished observations). Extensively unwound DNA serves as a substrate for primase; once primers are formed, DNA polymerase III holoenzyme initiates synthesis of DNA.

In the scheme presented above, HU appears to act as an accessory protein. Since it is not required for the binding of either DnaA or DnaB (Funnell et al., unpublished observations), a reasonable hypothesis is that HU affects the ability of DnaB to open a bubble in the DNA.

An in vitro system for examining initiation of replication from the bacteriophage lambda origin has also been developed (88a). In this case HU appears to have an effect opposite to that described above for the chromosomal origin. In vivo and in a crude in vitro system lambda replication requires transcription for activation. However, in reaction mixtures containing nine purified phage and bacterial proteins, replication occurs in the absence of transcription. Suspecting that transcription might remove an inhibitor, McMacken and his colleagues examined *E. coli* extracts for an inhibitor of lambda replication. His group found that HU inhibits replication, apparently by preventing formation of a prepriming complex (88a).

IHF

The most fully documented function for a histonelike protein is the strong stimulatory role played by IHF in site-specific recombination, a role first demonstrated in vitro for recombination of bacteriophage lambda (65). IHF is also the best understood of the histonelike proteins at the genetic level. Mutations have been available since 1977 in the genes encoding IHF (93, 154), and they have now been mapped, cloned, and sequenced (33, 89, 92). IHF-α is encoded by *himA*, which maps at 38 min on the standard genetic map of the *E. coli* chromosome (89, 95, 96, 97). IHF-β is encoded by *hip* (also called *himD*), which maps at 21 min (33, 35, 95, 96, 97). Mutations in *himA* or *hip* block site-specific recombination in several phage systems (lambda, φ80, and P2) and decrease the reversion frequency of mutations generated by insertion of Tn*5* and Tn*10* (94). Wild-type alleles of both genes are dominant over mutant alleles, consistent with the idea that the gene products are positive effectors of site-specific recombination (64, 94).

It is important to point out that IHF is only an accessory protein for recombination: in the bacteriophage lambda system Int alone can catalyze recombination, and mutations in *int* have been obtained which overcome the defect imposed by *himA* and *hip* mutations (77, 94). Nevertheless, in the wild-type situation IHF is an important factor and is required for in vitro recombination (100). Binding sites for both Int and IHF have been located within the *attP* recombination site (Fig. 5), and mutations have been generated in vitro within some of the sites. Mutations in the IHF-binding site designated H1 reduce the binding affinity of IHF for that site and reduce site-specific recombination both in vitro and in vivo (38). Since mutation of site H1 does not affect the binding to IHF to the two other sites, it is unlikely that cooperative binding among the sites is a major factor in IHF function. Mutation of each of the other sites also reduces recombination, leading to the conclusion that all three sites are necessary for efficient recombination. It has been suggested that the three sites are not functionally equivalent (38), since mutations in the sites differ in their relative effectiveness depending on whether recombination is measured in vitro or in vivo and whether recombination is integrative or excisive.

Support is now building for the idea that IHF participates with Int in wrapping the *attP* recombination site into a nucleosomelike structure called an "*attP* intasome." Electron microscopic observations indicate that Int and *attP*-containing DNA form complexes that are about 14 nm in diameter, contain about 230 bp of DNA, and involve four to eight molecules of Int (5, 29). Although IHF has little effect on the gross morphology of these complexes, it does seem to narrow the range of nucleotide sequences where the complexes are found (5). The conclusion that the complex wraps DNA is derived primarily from topological considerations. When substrates for intramolecular site-specific recombination are constructed such that the recombination sites are inverted, close together, and on a relaxed DNA, half of the products are simple trefoil knots (111). Electron microscopic

TABLE 4. Genes tested for effects of *himA* or *hip* mutations on expression

Expression in *himA* and/or *hip* mutants	Genes	Reference(s)
Increased	*himA*	96
	hip	96
Decreased	*gyrA*	42
	Lambda cII	58, 104
	ilvB	34
	ilvGEDA	34
	Mu early transcription	46, 48, 70
No change	*lac*	96
	gyrB	42

studies show that all of the knots are identical and that each contains three nodes of positive signs (52). Knots of this type are most easily explained if, prior to recombination, DNA is wrapped by Int and IHF in the same sense as found in nucleosomes. If this is the case, then negative supercoiling could stimulate recombination by facilitating the formation of wrapped structures.

Negative supercoiling is known to stimulate integrative recombination both in vitro (44, 98) and in vivo (66). Experiments with Int-h, the mutant form of Int capable of recombination in the absence of IHF, suggest that IHF action may be related to the stimulatory effect of supercoiling: recombination mediated by Int-h is stimulated by negative supercoiling only if IHF is also present (77). Supercoiling does not greatly alter the contacts IHF and Int make with their specific binding sites on *attP* as judged by methylation patterns following treatment with dimethyl sulfate (116). However, when excess competitor DNA was added to reaction mixtures, an increased affinity of Int for the P1 site was observed when the DNA was supercoiled (116). A similar phenomenon was seen for the P'3 site, but the difference between linear and supercoiled DNA was less than that observed with P1. In both cases the presence of IHF was required to observe the preference for supercoiled DNA. An important observation with this system is that the effect of supercoiling and IHF on Int binding is not observed if the P1 and P'3 sites are removed from their normal location in *attP*. Such a finding is consistent with supercoiling and IHF promoting the wrapping of *attP* by Int.

Packaging of phage DNA is another process in which IHF participates. In contrast to bacteriophage lambda, the lambdoid phage 21 is unable to grow in *himA* and *hip* mutants. Phage mutations called *her* suppress the *himA* and *hip* mutant phenotype, and *her* mutations map in the N-terminal region of the gene encoding the small subunit of terminase (32). This region of terminase is responsible for binding to phage 21 DNA at the *cos* site and is probably important in DNA packaging. Three potential IHF-binding sites occur in the phage 21 *cos* site, and in vitro studies confirm that IHF stimulates phage 21 packaging (32). In a related case, a mutant of lambda, *cos-154*, fails to grow in *himA* and *hip* mutants (3). The mutation *cos-154* is a single-base change near the left cohesive end of lambda that appears to prevent phage packaging in the presence of *himA* and *hip* mutations. These two phage studies can be related if IHF functions as an accessory protein for terminase-catalyzed cleavage of DNA at *cos*, a reaction that may be homologous to Int-mediated recombination (3). According to this hypothesis, lambda terminase would not normally require IHF, but if the terminase recognition site is modified by mutation (*cos-154*), the proper complex formation with IHF becomes crucial. As with recombination, IHF action may be related to supercoiling: plating efficiency of *cos-154* in a *himA gyrB* double mutant is lower than in either single mutant (3). In this context one would predict that the terminase-*cos* interaction of phage 21 is weaker than that of lambda and normally requires IHF to properly orient the DNA for cleavage.

Similar cases are also emerging in the area of transposition. An in vitro system has been developed for transposition by Tn*10*, and IHF is one of the host factors required for this transposition (Morisato and Kleckner, personal communication). In another example IHF has been shown to bind to sites located at each end of the insertion element IS*1* (Gamas et al., personal communication). Thus IHF appears to participate in a variety of types of site-specific recombination.

The *himA* and *hip* genes are not essential for the survival of *E. coli* (64), and their physiological function is not known. One emerging hypothesis is that they are involved in large control networks. The *himA* gene is located in the phenylalanyl transfer RNA synthetase operon, suggesting that *himA* expression may be affected by the growth factors that influence that operon (89, 92, 101). As with other synthetase genes, *pheS* and *pheT* increase expression as growth rate increases. The *himA* gene may also be part of the SOS pathway, although the level of repression of *himA* by the *lexA* gene product is small (96). The two genes upstream from *himA*, *pheS*, and *pheT*, are not influenced by *lexA* (92), indicating that the control of *himA* expression may be complex. Expression of *himA* may itself be involved in modulating other cellular activities including DNA supercoiling. Mutations in *himA* lead to a fourfold reduction in *gyrA* expression and lowered gyrase activity when the mutation is present in a strain also containing a *gyrB* (*himB*) mutation (35, 42). Since changes in supercoiling are known to affect the expression of a number of genes (for list, see reference 26), it is possible that IHF levels indirectly affect the expression of many genes.

IHF also appears to directly affect the expression of certain genes (see Table 4 for list of genes examined for effects of *himA* and *hip* mutations). In the case of the early genes of bacteriophage Mu, *himA* and *hip* mutations lead to reduced transcription from the early promoter, and they can be suppressed by promoter-up mutations that map in the Pribnow box of the early promoter (46–48, 70). Thus it appears that IHF plays a positive role in initiation of transcription in Mu. The control region for the repressor and early genes of Mu is sketched in Fig. 6. Three repressor binding sites (O_1, O_2, and O_3) are found between the repres-

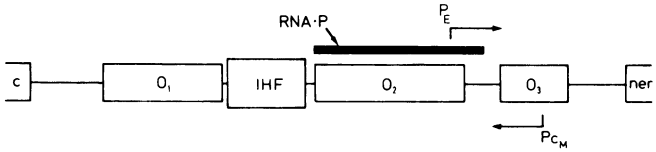

FIG. 6. Control region of the early promoter and repressor genes of bacteriophage Mu. Transcription of the early genes originates at p_E and progresses rightward, while that of the repressor gene begins at pc_M and progresses leftward. Repressor-binding sites are shown as boxes designated O_1, O_2, and O_3. The IHF-binding site falls between O_1 and O_2. The region protected by RNA polymerase binding is shown as a thick solid line. The repressor gene is labeled *c* and the first gene of the early operon is labeled *ner*. The figure is redrawn from reference 70.

sor gene (*c*) and the first early gene (*ner*), and inserted between two of them is an IHF-binding site. Footprinting analyses show that the IHF-binding site is immediately upstream from the RNA polymerase-binding site for the early gene promoter (p_L) (70). When transcription is measured in vitro, the presence of IHF leads to a three- to fivefold increase from p_L and an 80% decrease from pc_M, the promoter for the repressor gene (70).

IHF appears to function in a different way in the regulation of the *cII* gene of phage lambda. This gene is one of the key elements in the decision between lytic growth and lysogeny, and when the *cII* product is abundant, lysogeny is favored. Mutations in *himA* or *hip* lower the production of *cII* (60, 110). Since in these mutants *cII* synthesis is lowered relative to the product of a downstream gene on the same transcript, it was suggested that IHF is a positive effector of a posttranscriptional event (58). IHF binds to the *cII* gene, protecting a 33-bp region 25 bp upstream from the translation initiation codon of the gene (15). Between the IHF-binding site and the initiation codon is a site required for IHF to stimulate translation, as judged by gene fusion experiments (85). It has been suggested that binding of IHF might alter the dissociation of messenger RNA from the DNA template and thereby influence RNA structure and ribosome binding (85). The IHF-binding site also overlaps with the tR1 terminator, and in vitro the presence of IHF increases transcription of *cII* by two- to threefold (107). Perhaps antitermination of transcription is still another way in which IHF affects gene expression.

The products of the *himA* and *hip* genes also regulate each other. When expression from *himA* is monitored by using a *lacZ* fusion or when synthesis of IHF-α and IHF-β is measured by incorporation of radioactivity into protein spots displayed by two-dimensional gel electrophoresis, expression of both *himA* and *hip* appears to increase in *himA* or *hip* mutants (96). Thus it appears that the product of each gene negatively regulates its own expression as well as expression from the other. Since a putative IHF-binding site overlaps the promoter of *hip*, IHF may regulate synthesis of its own beta subunit by competing with RNA polymerase for binding to the *hip* promoter (92). How regulation of the *himA* gene occurs is still unclear since *himA* is the third gene in the *pheST* operon (89, 92). There is no evidence that the product of either the *himA* or the *hip* gene is required for expression of the other: biologically active products from each gene can be synthesized in the absence of the other (95).

The possible role of HU in IHF function is another aspect that is now being addressed. Initially the bacteriophage lambda recombination system provided no support for functional relatedness between the two proteins: HU did not substitute for IHF (100), HU showed no specific protection pattern when bound to *attP* (15), and antibody directed against HU did not inhibit recombination (100). However, a mutation in the H′ binding site for IHF in *attP* allows more recombination in vivo than in vitro, and crude extracts from *himA* and *hip* mutants contain a factor that stimulates in vitro recombination of the mutant DNA when IHF and Int are present (38). Under similar conditions, HU also stimulates recombination by IHF and Int (cited in reference 38). Another example is found in the Tn*10* transposition system developed by Morisato and Kleckner. This system requires host factors, one of which is IHF. Additional stimulatory factors are found in IHF cells, and purified HU has a modest stimulatory effect, both alone and in combination with IHF (Morisato and Kleckner, personal communication). Still another example of relatedness may emerge from studies of initiation of DNA replication. It has recently been found that replication of plasmid pSC101 in vivo is blocked by mutations in the *himA* and *hip* genes (37a). Replication of this plasmid is *dnaA* dependent, and a region of DNA homologous to the IHF consensus binding sequence has been found between the DnaA and RepA binding sites (37a). Although the effect of the *himA* and *hip* mutations can be explained in several ways, one interesting possibility is that IHF participates with DnaA, RepA, and the origin of replication to form a specific structure needed for initiation of replication. As pointed out above, HU may have a similar role in *dnaA*-, *oriC*-dependent replication. Taken together, these observations suggest that IHF and HU may be functionally related.

Other Histonelike Proteins

firA is the only other known locus affecting a histonelike protein. As mentioned above, this gene, which maps at about 4 min on the *E. coli* map between *dapD* and *polC* (78), confers sensitivity to rifampin in Rif^r *rpoB* mutants. The discovery of a temperature-sensitive allele suggests that *firA* may be an essential gene, and physiological studies indicate that the product of the gene is involved in transcription (79).

Protein H1 also appears to affect transcription. Although the initial studies showed that H1 stimulates transcription (17, 18), the most detailed study with a single promoter (*lacL8* UV5) reveals a case in which H1 decreases the rate of initiation of transcription. Analysis of abortive initiation reactions suggests that the protein slows the isomerization step in initiation but has little effect on the binding of RNA polymerase to the DNA (140).

In addition to the possible functions of histonelike proteins described above, in some organisms these proteins may serve to stabilize DNA from denaturation under extreme environmental conditions. The clearest example of this phenomenon is the HTa protein of *T. acidophilum*, an organism that grows at very high temperatures and low pH. As mentioned above, the HTa protein can raise the melting temperature of DNA by 40°C.

CONCLUDING REMARKS

Five bacterial proteins have been called histonelike because some of their biochemical properties resemble those of eucaryotic histones. However, no clear biochemical definition of bacterial chromatin has been obtained, and we still do not know whether any of these five proteins participate in condensation of large regions of the bacterial chromosome. Nevertheless, a function is emerging for two of the proteins, HU and IHF: they appear to act as accessory proteins in processes involving recognition of specific nucleotide sequences. Currently, no general statements can be made about the three other proteins, H, H1, and FirA.

Both HU and IHF appear to participate in wrapping DNA. It has been suggested that DNA condensation and wrapping into precise structures is a way to ensure that specific nucleotide sequences are recognized during site-specific recombination and initiation of DNA replication (29, 30). We now suggest that there are at least five elements involved in precise recognition of nucleotide sequences: (i) the nucleotide sequence, (ii) a specific protein that recognizes the sequence (Int, DnaA, Hin, etc.), (iii) an accessory protein involved in DNA wrapping (HU, IHF), (iv) negative superhelical tension in the DNA, and (v) special DNA structures popularly known as bent DNA. Discussion of the

TABLE 5. Functional regions containing bent DNA

Region	Reference
Phage lambda *att*	123
Phage φ80 *att*	82
Phage P22 *att*	82
Plasmid pT181 *ori*	69a
Plasmid R6K *ori* (initiator protein induced)	99
Phage lambda *ori*	158
SV40[a] *ori*	130
lac promoter (CAP[b] protein induced)	156
Upstream from *hisR* promoter	7
Mu early promoter	P. Higgins, personal communication

[a] SV40, Simian virus 40.
[b] CAP, Catabolite gene activator protein.

first two elements is beyond the scope of this review. Combination of genetic and biochemical analyses now establishes that the histonelike protein IHF serves as an accessory protein in site-specific recombination by bacteriophage lambda, probably by assisting in the bending and wrapping of the *attP* recombination site (see Fig. 6 in reference 116). A comparable mechanism may operate in transposition and phage packaging when transposase and terminase, respectively, replace Int. Biochemical analyses are pointing toward a similar role for HU in other types of site-specific recombination and in initiation of DNA replication. Superhelical tension in the substrate DNA enhances most examples of site-specific recombination and initiation of replication that have been studied in vitro. Bent DNA is characterized by an anomalously slow electrophoretic mobility of restriction fragments, a behavior that is more pronounced when the bend or curvature is near the middle of the fragment (156). Bent DNA is associated with several *att* sites and origins of replication (for list and references, see Table 5). Bent DNA has also been found associated with promoters (Table 5), and it may be that HU and IHF, along with superhelical tension and DNA bending, facilitate the DNA looping postulated to be important in gene regulation (for review of looping, see reference 114).

Understanding other aspects of the biology of the histonelike proteins has been difficult. Even with the availability of the *himA* and *hip* mutations, it is still unclear what host function IHF serves: it must not perform an essential function in *E. coli* since *himA* and *hip* mutants are viable. IHF may serve as a sensor of internal environmental conditions, and perhaps it transmits that information to the genetic apparatus by binding near specific genes, such as *gyrA*, to influence DNA topology and chromosome structure. Bacteriophage lambda may exploit IHF to sense the intracellular environment (11). In the absence of IHF, integration does not occur, nor is repressor synthesized (58, 94); thus low concentrations of IHF would favor lysis.

DNA superhelical tension is clearly an important aspect of bacterial chromatin structure. Topoisomerases actively introduce and maintain the tension, and perturbation of their activities leads to disruption of a number of chromosomal processes in parallel with the loss of titratable DNA supercoiling. The intracellular level of superhelical tension is probably about half that measured in deproteinized DNA (6a); presumably histonelike proteins wrap the DNA in such a way that about half of the tension is removed (109). We now need to know if the histonelike proteins participate in regulatory processes through the effects their binding to DNA could have on local levels of superhelical tension.

The lack of good cell fractionation procedures for the isolation of bacterial chromatin has impeded progress in examining the possibility that histonelike proteins play a general role in chromosomal DNA packaging. Bacterial nucleoids are not separated from the cytoplasm by a membrane as are nuclei in eucaryotic cells, so the putative chromatin cannot easily be isolated from cytoplasmic material. The bacterial nucleoid can be extracted from cells, but to maintain it in a compact, nonviscous conformation, high concentrations of counterions must be added to cell lysates (for review, see reference 27). It is likely that high counterion concentration removes some of the putative chromatin proteins from the DNA. Although gentle cell lysis at low salt concentration has revealed microscopically distinct nucleosomelike structures (51, 87), these are labile. Cell fractionation procedures have not produced bacterial DNA wrapped by HU or any other protein. Thus it has not been firmly established that HU is a major structural component of bacterial chromatin.

Another problem has been the absence of mutations in the genes encoding the HU class of protein. As a result, hypotheses concerning function for these proteins rest on biochemical analyses. We are left with the possibility that some of the effects of HU on assays involving initiation of replication or site-specific recombination may be fortuitous. HU is closely related to IHF, and it is important to use *himA* and *hip* mutations to rule out the possibility that in vitro HU is participating in a reaction which in living cells actually involves IHF. Nevertheless, we expect that the availability of these functional assays will speed progress toward understanding the HU proteins. Since the HU genes have now been cloned (62; Imamoto, personal communication), it should be only a matter of time before mutations constructed in vitro are available to establish physiological functions for HU.

We now need to bridge the gap between eucaryotic and procaryotic chromatin and understand why the two structures are seemingly so different. A major difference between bacteria and higher organisms is that virtually all bacterial genes are available for transcription while the majority of the genes in higher organisms are not. One attractive idea is that bacterial chromatin is similar to the minor class of eucaryotic chromatin which contains active and activatable genes. If so, we would expect the effect of superhelical tension on eucaryotic genes to be similar to its effect on bacterial genes. Reports are now beginning to appear which are consistent with this being the case (69, 151). The type of DNA wrapping and looping observed in procaryotic systems may turn out to play an important regulatory role in eucaryotic systems in which two nucleotide sequence elements must be brought together, and the *hin*-HU system (60a) may serve as a useful model for enhancer action. As the characteristics of bacterial chromatin structure become better defined, it will be interesting to see which properties are shared by transcriptionally active regions of eucaryotic chromatin.

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