Histone-like Proteins and Bacterial Chromosome Structure

David E. Pettijohn
From the Department of Biochemistry, Biophysics/Genetics, University of Colorado Health Sciences Center, Denver, Colorado 80262

Prokaryotic cells synthesize a set of small, usually basic proteins that bind DNA and are collectively called histone-like proteins because their biochemical properties resemble eukaryotic histones. Whereas the eukaryotic histones have a well defined function in packaging DNA through restraining left-handed toroidal DNA supercoils in nucleosomes, the functions of the prokaryotic histone-like proteins are not yet clear. However, recent studies of the interactions of certain histone-like proteins with DNA and genetic studies of cells that are mutated in genes coding for these proteins are beginning to suggest functions for some of the proteins. These findings and the results to prokaryotic chromosome structure will be briefly reviewed here.

At this time five different types of bacterial proteins are considered to be histone-like. These include: 1) the Hu protein (for review see Drlica and Rouviere-Yaniv, 1987), which is a small dimeric protein that in Escherichia coli is composed of subunits of M, 9,535 and 9,225 named Hu-a and Hu-b, respectively, also called Hu-2 and Hu-1, respectively, and coded by the genes hupa and hupb, respectively (many other bacterial species have only a single gene and a single type of Hu subunit); 2) integration host factor (IHF) (Nash and Robertson, 1981), a basic protein having substantial amino acid sequence homology with Hu, apparently composed of two subunits IHF-a and IHF-b, having M, 11,224 and 10,581, respectively, and coded by the E. coli genes himA and himB, respectively; 3) protein H1 (Spasiky et al., 1984), a neutral protein existing in three different forms H1a, H1b, and H1c having different isoelectric points, but the same M, 15,500; 4) HLP1 (Lathe et al., 1980), a protein of M, 17,000, which is apparently the product of the E. coli firA gene; 5) protein H (Hubscher et al., 1980) of M, 28,000, cross-reacting with antibodies specific for eukaryotic histone H2A. Also, bacteriophages can code for specific proteins having homology to Hu protein (Greene et al., 1984) and can make proteins that couple to host cell Hu and apparently modify it functionally. Each of these histone-like proteins has a relatively high intracellular concentration, which in E. coli ranges from that of H and Hu which are the most abundant, roughly 120,000 and 60,000 monomers per cell, respectively, to the least abundant H1 and IHF estimated at about 14,000 to 20,000 copies or less per exponentially growing cell. It is important to recognize that some of the figures for abundance could be underestimated, since they are calculated from recoveries after purification. The Hu proteins, which are the most thoroughly studied, are highly conserved in different prokaryotes and are found in eubacteria, archaeobacteria, blue-green algae, and also plant chloroplasts. Details of the evolutionary relationships, amino acid sequences, other structural details, and the various nomenclatures used in the literature in referring to the histone-like proteins were nicely summarized in recent reviews (Gualerzi et al., 1986; Drlica and Rouviere-Yaniv, 1987) and will not be discussed here.

HU Protein and DNA Wrapping

Recent findings are revealing an involvement of Hu in the coiling or wrapping of specific DNA sequences in different regions of the chromosome. It has been known for some time that purified Hu protein can decrease the linking number of bound DNA sequences, and DNAs become negatively supercoiled as a result of having bound Hu (Rouviere-Yaniv et al., 1979). Several features of this interaction are analogous to the histone-DNA interaction of eukaryotic nucleosomes, which led to the proposal that Hu can form nucleosome-like structures. Indeed, complexes of Hu and SV40 DNA (1:1 mass ratio) fixed by glutaraldehyde were observed by electron microscopy to consist of about 14 beaded structures per DNA circle. Nuclease digestion studies of the Hu-DNA complex have also shown that the DNA in the complex is cut at regular 8.5-base pair intervals, qualitatively analogous to the manner in which DNA on the surface of nucleosomes is cut by certain deoxyribonucleases (Broyles and Pettijohn, 1986).

Hu seems to have little sequence specificity, and it is perhaps not surprising that it may be involved in several different DNA-protein interactions that require wrapping or coiling of specific DNA sequences. One example is the stimulatory effect of Hu on the initiation of DNA replication in vitro at the E. coli replication origin (Dixon and Kornberg, 1984). In this in vitro system a prepriming complex is formed, consisting of a supercoiled plasmid DNA containing the chromosomal origin sequence oriC associated with the dnaA, dnaB, dnaC, and HU proteins. These proteins assemble to produce a large complex on which the oriC sequence seems to be wrapped around the dnaA protein, as indicated by nuclease digestion studies (Fuller et al., 1984). Hu has been localized at or near this complex (Funnell et al., 1987). Upon addition of single-strand DNA binding protein plus DNA gyrase, the replication bubble forms and this structure becomes the substrate for the E. coli primase, leading eventually to the bidirectional initiation of DNA replication by DNA polymerase III. Hu is not absolutely required for this process but acts as a stimulatory accessory protein.

Another example is the participation of Hu in the binding of lac operator sequences by the lac repressor (Flashner and Gralla, 1988). When the lac repressor binds specifically to its operators it distorts the DNA and brings into close proximity two operators from among the three related sequences O1, O2, and O3, probably by DNA looping or coiling (Kramer et al., 1988). Using a DNA footprinting method to assay the specific binding of lac repressor, Flashner and Gralla (1988) demonstrated that the binding to operator O1 in linear DNA was enhanced about 20-fold in the presence of Hu. In addition they found that specific DNA recognition by catabolite activator protein, which also seems to require bending or coiling of the DNA, is strongly facilitated by Hu. By contrast, the specific recognition by trp repressor of arnH DNA, which does not appear to require structural distortion of the DNA, is
either unaffected by HU or slightly inhibited. It was proposed that HU is a general facilitator of protein-DNA interactions that require certain kinds of distortions of the DNA binding sequence. Further support for this idea came from studies of the effects of BaCl₂ on the same protein-DNA interactions. Ba²⁺ is known to stabilize bent DNA forms, and it was shown that this ion also facilitates the same protein-DNA interactions as HU (but to a lesser extent) and like HU inhibits the trp repressor binding.

Other protein-DNA interactions can also involve HU as an accessory protein. HU was discovered as a factor that stimulates transcription in vitro of the bacteriophage λ template, but subsequent studies have shown that HU can either stimulate or inhibit transcription, depending on the template and other conditions (for review see Drlica and Rouviere-Yaniv, 1987). Several in vitro systems for site-specific DNA re-arrangements also require HU for maximal activity. Examples are: 1) the DNA inversion reaction associated with flagellar phase variation in Salmonella in which there is a switching of the orientation of a promoter required for transcription of one flagellin gene and repression of another. This reaction is stimulated about 10-fold by HU, but the degree of stimulation differs depending on the separation of a nearby enhancer (Johnson et al., 1986), leading the investigators to postulate that the role of HU may be in positioning different parts of the protein-DNA complex possibly by DNA bending. 2) HU is also required for transposition by bacteriophage Mu (Craigie et al., 1985) and may make up part of the transpososome (Surette et al., 1987).

In most, if not all, of the above interactions involving different proteins having different DNA sequence specificities, the optimal amounts of HU are far less than the amounts required to saturate the DNA. This indicates that the HU protein can be localized at DNA sequences where the other proteins specifically bind. Since other studies have shown that HU by itself has little DNA sequence specificity, its localization implies that it may bind cooperatively with the other proteins that have sequence specificity. The reversible nature of HU-DNA interactions (discussed below) may greatly assist in a sorting process that eventually leads to its localization.

**Mutations in hupA and hupB Genes**

The hupA and hupB genes coding for HU have been cloned and sequenced (Kano et al., 1985, 1987), and recently clones of these sequences have been used to produce and select both single and double deletion or insertion mutants of E. coli. Careful Southern and Northern blot analyses of nucleic acids from these mutants demonstrated that the genes and their gene products were absent in the deletion lines and altered in the insertion mutants.

As was observed earlier (Storts and Markovitz, 1988), a mutation in either the hupa or hupb gene alone has no observed effect on the growth or general regulatory properties of the mutants. Apparently, HU homodimers can perform the functions of HU, as they also do in species that normally have only one type of HU gene. On the other hand, the mutants in which both HU genes were deleted or otherwise mutated were altered phenotypically but, interestingly, were still able to grow. These mutants cannot support fertility plasmid F′gal or a variety of mini-F plasmids, and the results indicate that ori-2-dependent DNA replication was defective. Likewise, bacteriophage Mu was unable to carry out replicative transcription in the double mutant. Unlike wild-type cells, the double mutants are killed by heat or cold shock, form abnormally filamentous cells containing abnormal nucleoids at any temperature, and about ⅓ to ⅔ of the cells in a culture segregate without DNA. The observation that, in spite of these abnormalities, the double mutant cells can form colonies and grow indicates that HU is dispensable. However, it is possible that compensatory changes in different histone-like proteins or other proteins permit survival. These studies indicate that the transcriptional rates of the HU genes are autoregulated by the intracellular concentrations of the different HU subunits and that the concentration of HU may also influence the levels of other histone-like proteins, such as IHF. Such coordinated regulation could compensate for loss of HU, but none of the studies yet provide evidence for or against this possibility.

**Functions of Other Histone-like Proteins**

The role of IHF in uninfected cells is not known; however, several in vitro systems for site-specific recombination involving E. coli bacteriophage DNAs are strongly stimulated by IHF. This histone-like protein participates in integrative or excisive recombination of λDNA and also other phage DNAs, in transposition by Tn10, and also in the packaging of DNA in phage heads (for review see Drlica and Rouviere-Yaniv, 1987). Mutations in the E. coli genes himA or hip, coding for IHF, block site-specific recombination in the φ80, P2, and λ systems, but like the HU mutants do not block cell survival. The products of the himA and hip genes are autoregulated (Miller et al., 1981) and also regulate each other, resembling the situation with HU genes. Recent studies have further indicated possible coordinate interactions between HU and IHF, in that himA mutants of Salmonella are killed when the hupa gene is overexpressed by introduction of the cloned hupa. These results again indicate the possibility that regulatory changes in other genes may partially compensate for mutational loss of a histone-like protein.

As might be expected, given its structural similarity to HU, IHF may have functional similarities to HU. IHF seems to participate with the λ int protein in forming nucleosome-like structures in which the DNA is wrapped in left-handed toroidal supercoils (Pollock and Nash, 1983). However, unlike HU, IHF has a DNA binding specificity and recognizes the consensus sequence Pyr-AANNNNTTGAT-A/T (Leong et al., 1986).

The functions of the other histone-like proteins are still poorly defined. As judged by the concentrations of monovalent salt required to disrupt DNA binding, H1 protein binds stronger to DNA than any of the other proteins. The sedimentation rate of a bound plasmid DNA is increased more than predicted from the mass change, implying that the DNA is compacted; however, studies of the effects of topoisomerase I on the linking number of supercoiled DNA bound to H1 have indicated that the H1 protein does not supercoil the DNA like HU or IHF (Spassky et al., 1984). The gene for protein H-NS, which is likely identical to one of the forms of IHF, blocks site-specific recombination in the φ80, P2, and λ systems, but like the HU mutants do not block cell survival. The products of the himA and hip genes are autoregulated (Miller et al., 1981) and also regulate each other, resembling the situation with HU genes. Recent studies have further indicated possible coordinate interactions between HU and IHF, in that himA mutants of Salmonella are killed when the hupa gene is overexpressed by introduction of the cloned hupa. These results again indicate the possibility that regulatory changes in other genes may partially compensate for mutational loss of a histone-like protein.

**Other Histone-like Proteins of Interest**

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**Minireview: Histone-like Proteins**

Physical-chemical studies of the isolated bacterial nucleoid and related studies of the nucleoid in vivo have revealed two general levels of DNA organization (reviewed in Pettijohn and Sinden, 1985). (a) There is a long-range structure that involves folding of the single DNA molecule of the chromosome and a set of constraints that limit the rotation of the double helix. These restraints result in the segregation of the DNA molecule into about 43 chromosomal domains (about 100 kilobase pairs per average domain). The DNA torsional tension (or the DNA supercoiling) can be relaxed in one domain while not affecting tension or supercoiling in other domains. (b) There is a short-range structure leading to negative DNA supercoiling, corresponding to a specific linking number deficit of $\gamma = -0.05$ to 0.08.

The possible involvement of histone-like proteins in organizing these regular elements of bacterial chromosome structure has been actively considered for some time. It was known, for example, that the DNA superhelical density estimated from the above linking numbers is close to the density found in eukaryotic DNAs, where most if not all of the supercoils are organized in nucleosomes. Thus it seemed possible that the negative supercoils in prokaryotic chromosomes could likewise be stabilized in nucleosome-like structures involving one or more types of histone-like proteins. Studies of the isolated nucleoids provided little evidence for nucleosomes; however, it was known that the method for isolating the cleanest nucleoids used conditions that dissociated many histone-like proteins including HU. When other isolation methods were used that better stabilized protein-DNA interactions, HU and other histone-like proteins were associated with the nucleoid, and nuclease digestion of protein-DNA complexes showed that 120-base pair DNA fragments were protected that had bound HU and one other histone-like protein. There was on average one monomer of protein per 150 to 200 base pairs of DNA; however, regular ladders of protected fragments such as those found with eukaryotic nucleosomes were not observed (review Pettijohn and Sinden, 1985; Drlica and Rouviere-Yaniv, 1987). Moreover, examination of these isolated chromosomes by a variety of techniques previously used to define eukaryotic nucleosomes provided no evidence for regular arrays of stable nucleosomes.

Subsequent studies of the interaction of purified HU with DNA showed that the dissociation half-life is quite short (~90 s in solutions containing 0.05 M NaCl) and that brief digests with high concentrations of nuclease (periods less than the half-life) produced regular protection ladders more similar to that of nucleosomes (Broyles and Pettijohn, 1986). It was also demonstrated that the DNA in these rapidly exchanging complexes is restrained in a form having linking number deficits characteristic of negatively supercoiled DNA. Thus, it seemed possible that nucleosome-like structures do exist in vivo, but they spontaneously dissociate or disorganize when cells are disrupted by any of the common methods for isolating protein-DNA complexes. To explore this possibility methods were needed for examining in vivo the supercoiling of DNA and its possible organization.

Early studies using trimethylpsoralen photoaffinity probes showed that the DNA in living bacterial cells is maintained in a state of negative torsional tension (i.e. the double helix is underwound) while DNA in eukaryotic cells had no detectable tension averaged over the entire genome (reviewed in Pettijohn, 1989). This result supported earlier findings with isolated eukaryotic chromatin that all or nearly all of the supercoils in eukaryotic systems were restrained in nucleosomes so that there was little net torsional tension in the DNA, but the result also indicated that a significant fraction of supercoils in bacterial DNAs was not restrained in vivo. In another approach the E. coli plasmid F' was nicked by gamma irradiation in vivo and allowed to repair under conditions where DNA gyrase was inhibited from introducing any supercoils that were relaxed (Pettijohn and Pfenninger, 1980). It was found that only about 1/4 of the DNA supercoils in isolated control F' DNA could be relaxed while the DNA was nicked in vivo. This suggested that about half the supercoils in vivo are restrained in nucleosomes and the other half are not. Later studies of the amount of supercoiling required to convert DNA inverted repeat sequences to cruciforms in vivo (reviewed in Lilley, 1986) and other measurements of the eukaryotic nucleosome-like structures of Int-mediated recombination (Bliska and Cozzarelli, 1987) also led to the conclusion that negative supercoils are present in vivo but account for only about 40% of the superhelical density of the isolated deproteinized DNAs. These results are all compatible with the view that there are nucleosomes or some topologically equivalent constraint in bacterial chromosomes.

It remains uncertain if any of the histone-like proteins are responsible for the organization of these nucleosome-like structures. While the observations reviewed above show that HU and IHF can contribute to the coiling or wrapping of DNA at specific loci in the chromosome, and in vitro these proteins can supercoil DNA, it is not clear if they have a more general DNA packaging function. Indeed, recent studies of the distribution of HU protein using immunochemical staining of carefully preserved sections from E. coli suggest that this protein is distributed primarily on the surface of the nucleoid rather than throughout the chromosome (Bjornsti et al., 1986; Footnote 5). The surface is also the primary site recognized by antibodies specific for topoisomerase I and single-stranded DNA (presumably involved in transcription), which led Kellenberger and coworkers (Bjornsti et al., 1986; and Footnote 5) to suggest that the primary sites of DNA transcription are located there and that the major role of HU could be associated with transcription or the closely coupled translation of mRNA.

There is also no indication that histone-like proteins have any role in organizing the long-range structure of DNA in the domain substructure of bacterial chromosomes; however, no studies have really been directed at this possibility. DNA gyrase is a leading candidate for one of the proteins to be involved, based on the analysis of binding sites for this topoisomerase (Snyder and Drlica, 1979; Footnote 6) which agrees rather well in number and distribution with the sites expected to define limits of chromosomal domains.

It would seem that the developments described above have set the stage for the elucidation of the biological functions of the histone-like proteins. These interesting proteins are among the most abundant proteins found in bacterial cells, and it is somewhat surprising that their functions have been so illusive. While a role of two of these proteins in facilitating DNA wrapping or coiling at specific loci is emerging, one suspects that the final story is just beginning.

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