At several *E. coli* promoters, initiation of transcription is repressed by a tight nucleoprotein complex formed by the assembly of the H-NS protein. In order to characterize the relationship between the structure of H-NS oligomers in solution and on relevant DNA fragments, we have compared wild-type H-NS and several transdominant H-NS mutants using gel shift assays, DNase I footprinting, analytical ultracentrifugation, and reactivity toward a cross-linking reagent. In solution, oligomerization occurs through two protein interfaces, one necessary to construct a dimeric core (and involving residues 1–64) and the other required for subsequent assembly of these dimers. We show that, as well as region 64–95, residues present in the NH$_2$-terminal coiled coil domain also participate in this second interface. Our results support the view that the same interacting interfaces are also involved on the DNA. We propose that the dimeric core recognizes specific motifs, with the second interface being critical for their correct head to tail assembly. The COOH-terminal domain of the protein contains the DNA binding motif essential for the discrimination of this specific functional assembly over competitive nonspecific H-NS polymers.

H-NS is a DNA-binding protein involved in structurally organizing the nucleoid of prokaryotic cells. It is also involved in the regulation of many pathways, most of which are related to the response of the cell to environmental changes (1, 2). In some well characterized cases, it exerts its action at the level of transcription, either alone or in conjunction with its paralog StPa (3, 4). Both genetic and biochemical studies indicate that in this instance regulation of transcription is not mediated by a classical local interaction of H-NS with a canonical DNA sequence but rather that H-NS constitutes specific assemblies on the DNA that invade the promoter and prevent the formation of an efficient active complex between RNA polymerase and the DNA sequence (5–8). The extent of repression is also markedly sensitive to external conditions such as temperature (9), growth phase control (10, 11), or osmotic regulation (12–14). In some extreme cases, gene expression can be so severely restrained that it cannot be relieved by point mutations. In the case of the *bg1* locus in *Escherichia coli*, the ability of H-NS to polymerize along the DNA from an AT-rich crucial element appeared critical for silencing (15–18).

The formulation of a detailed mechanistic model for the action of H-NS as a repressor has been hampered by several factors. The first one is the fact that *in vitro* H-NS binds to a number of DNA sequences, affecting the efficiency of transcription of promoters located on a segment even when it does not exert any critical role on their expression *in vivo*. Using different synthetic variants of the *gal* promoter control region, containing curved or straight inserted sequences, it was possible to distinguish functional from nonfunctional assemblies. Efficient repression requires first the building up of a substructure resulting from the binding of H-NS at the curved insert (the nucleation step) and then recruitment by a cooperative process of H-NS molecules bound at other strategic sites and in particular at the Pribnow box of the *gal* control region. Subsequent to these steps, H-NS polymerizes on the DNA fragment. If a straight sequence (instead of a curved one) is inserted upstream of the promoter, full coverage may still appear on the DNA *in vitro*. However, this requires high protein concentrations. In cases where H-NS is associated with promoter regions having straight sequences, it does not generally act as a repressor *in vivo*.

At certain promoters, the control of gene expression by a distant element requires more than an initial binding at a curved sequence and propagation from this site. For instance, for the *proU* operon of *Salmonella typhimurium*, the downstream element required for action at a distance, DRE (19, 20), cannot be efficiently substituted by any other curved DNA sequence (21). A specific nucleoprotein structure, which implies also a change in the topology of the whole DNA region, must be thus constructed for efficient repression to occur (19).

H-NS exists in solution under various oligomeric forms. For some time, the nature of the lowest oligomeric state of the H-NS was a matter of debate between a model where H-NS existed as a dimer or one in which it was a trimer. Recent biophysical and structural studies characterizing the precise interface between two monomers have resolved the controversy in favor of H-NS adopting a dimer configuration as the mini-
mum lowest state oligomer.\(^1\) Earlier biophysical experiments were also satisfactorily accounted for by using a model implying the existence of two coupled equilibria of the following type,

\[
4M \leftrightarrow 2D \leftrightarrow T
\]

\(4M \rightarrow 2D \rightarrow T\)  

**REACTION 1**

where \(M\) represents a monomer, \(D\) is a dimer, and \(T\) is a tetramer. The corresponding dissociation constants \(K_1 (10^{-7} \text{ m})\) and \(K_2 (10^{-15} \text{ m})\) are sensitive to ionic strength and to temperature.\(^2\) It is clear that a mechanistic model is needed to explain how the association interfaces involved in the oligomerization of H-NS in solution are rearranged during the formation of specific and nonspecific assemblies on a given DNA template.

To shed some light on these various issues, we have relied on the comparison between wild-type H-NS and proteins coded by dominant negative mutants of the *hns* gene, which are able to impair the normal function of the wild-type protein through the formation of wild type/mutant heterodimers or heteropolymers (23). These genetic studies as well as others led to the initial indications that H-NS consisted of two functional domains (29–26). Mutations between amino acid residues 90 and 121 reduce DNA binding activity. The latter is not affected by mutations in the amino-terminal domain between residues 12 and 65, which, however, remove repressor function. It was demonstrated that this NH\(_2\)-terminal part is responsible for protein-protein contacts (23, 25, 26). Three types of mutants were used in the present study: proteins modified either in the amino or the carboxyl terminus and a truncated protein containing only the first 64 amino acids of H-NS (H-NS 64A). All of these proteins have a dominant negative effect on the wild type protein in *vivo*, implying that they are still able to interact either with wild type monomers or with the DNA. These various proteins were first compared in their ability to bind specifically to curved and to noncurved DNA fragments occurring in natural sequences where the involvement of H-NS in repression had been previously tested: the dominant negative mutants have been selected on the basis of the derepression of the proU operon, and the two classes of mutations display differential effects at the proU and gal modified promoters (23). We therefore selected for more specific *in vitro* assays a portion of the proU promoter containing the negative regulatory element, NRE. The NRE, which is the equivalent of the DRE in *S. typhimurium*, is a region of about 500 base pairs downstream of the T\(^{70}\)-dependent promoter and overlapping the coding region for the first gene of the operon proV. NRE displays a region of moderate curvature centered around +196 with respect to this transcription start, and H-NS binding specificity is documented at this locus and at the upstream curved region of the promoter (20, 27–31).

The mode of association of various H-NS proteins to a proU linear DNA segment was analyzed by footprinting techniques. Relative binding efficiencies were compared and also related to our previous observations made on the association of WT\(^*\) H-NS protein with gal modified promoters (7). Finally, the association-dissociation equilibrium of wild-type H-NS in solution was analyzed under our experimental conditions and qualitatively compared with the behavior of several altered proteins.

1. Y. Yang, V. Bloch, E. Margeat, G. Herrada, C. Badaut, V. Arluison, B. Robert, S. Rimsky, and M. Kochoyan, submitted for publication.
2. The abbreviations used are: WT, wild type; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbo diimide.
order kinetic rate, which is determined by fitting the experimental curves with the equation,

$$I_{po} = e^{-k_{po}t} \quad (\text{Eq. 2})$$

where $I_{po}$ is the relative intensity (in arbitrary units) of each band detected on the gel, $k_{po}$ is the pseudo-first order rate of nonomer disappearance (in min$^{-1}$) during the reaction, and $t$ is the time in minutes of the cross-linking reaction.

**Analytical Ultracentrifugation**—Equilibrium sedimentation and sedimentation velocity experiments were performed at 20°C in a Beckman Optima XL-A ultracentrifuge using an AN60-titanium Four-Holes rotor and a cell with two-channel center pieces (path length 12 mm). Prior to centrifugation, purified WT, L26P, and Δ64 proteins were dialyzed at 4°C against 50 mM Tris-HCl, pH 6.8, containing 0.1 mM dithiothreitol and 500 mM NaCl (WT and L26P) or 200 mM NaCl (Δ64). The final concentration of H-NS proteins was 50 μM. Sedimentation velocity experiments were performed at 30,000 and 40,000 rpm for the WT and L26P proteins and 60,000 rpm for Δ64. Equilibrium sedimentation experiments were performed at 25,000 rpm. Radial scans of absorbance were taken at 280 nm for WT and L26P and 220 nm for Δ64 using diyalysis buffers as reference. Sedimentation velocity data were analyzed to provide the apparent distribution of sedimentation coefficients using the program DCDT$^+$ 1.12 (33). All measured sedimentation coefficients, $s^*_w$, were corrected into $s^*_w$, as expressed in Svedberg units using the program SEDNTERP program. Equilibrium sedimentation data were analyzed to yield weight average molecular masses using the program XL-AVX-I data analysis software 4.0 supplied by Beckman.

**RESULTS**

**Different Binding Modes of the Modified H-NS Proteins to the proU Promoter Region**

**Electrophoretic Mobility Shift Assays**

We used a gel retardation assay to characterize the ability of modified H-NS proteins to recognize specific DNA fragments (30, 34, 35). Digestion of a pBR322 plasmid using TaqI and SspI enzymes yields, in particular, a fragment carrying the bla promoter, which is known to contain curved sequences (36). Digested pBR was mixed in equimolar concentrations with a 372-bp DNA fragment carrying the E. coli control region of the proU promoter. The fragments were incubated with increasing concentrations of wild-type H-NS. As expected, the protein showed preferential binding for the fragment carrying the bla promoter, since a gel mobility shift was observed for the latter at an H-NS concentration of 0.2 μM (Fig. 1 I)). The proU fragment was also preferentially shifted at the same protein concentration, suggesting that the wild-type H-NS protein displays approximately the same affinity for the proU and the bla promoter regions.

The same experiment was then performed with the H-NS L26P, E53G/T55P, Y97C, P116S, and I119T proteins as well as with the H-NS Δ64 truncated protein. This last polypeptide did not display any detectable affinity for the DNA fragments (not shown). With all of the other altered proteins, significant changes in the overall pattern of mobility shifts with respect to the protein concentration were observed (Fig. 1, II–VI). The two proteins modified in their amino-terminal domain, H-NS L26P and E53G/T55P, retained the ability to recognize specifically the same fragments as the wild-type protein (although the H-NS L26P protein had lost some affinity for the bla promoter region). On the other hand, the H-NS Y97C, P116S, and I119T were impaired in their ability to clearly single out the bla and proU fragments at low protein concentrations. In the presence of the modified H-NS proteins, these fragments were no longer markedly shifted with respect to the pBR322-derived ones. As a consequence, the minimum concentration needed to observe shifted fragments was higher than for WT or NH2 terminus-modified proteins (1.5 μM for the H-NS Y97C as compared with 0.5 μM for the H-NS E53G/T55P; see Fig. 1, III and IV). In fact, the Y97C, P116S, and I119T proteins are modified in the carboxyl-terminal domain, which has already been suggested to be the H-NS DNA-binding domain (24). It is, however, worth noticing that these mutant proteins displayed some variations in their affinity for the fragments carrying the bla and proU promoters. The I119T mutant protein was the only one to have entirely lost its specificity for both fragments. For this mutant, the first DNA fragment of the set to be shifted by the protein was the largest one, as would be expected if this was total nonspecific binding. In contrast, the H-NS P116S, and more markedly the H-NS Y97C proteins still displayed some preferential affinity for the proU fragment. A gradation of effects was therefore observed in each of the two classes of dominant mutants considered. Nevertheless, the loss of recognition of specific DNA sequences was definitively localized in determinants present in the COOH-terminal domain of the protein.

**DNase I Footprint Experiments**

**Temperature Effect**—On the proU DNA fragment, Lucht et al. (30) observed the appearance of discrete DNase I footprints for protein concentrations in the 100 nM range at room temper-
Functional Oligomerization of H-NS

Similar experiments performed with the H-NS L26P led to similar conclusions (Fig. 3 (I), lanes g and h). These experiments strongly suggest that both the E53G/T55P and the L26P proteins, altered in their NH2-terminal domain, were still able to recognize nucleation sites or secondary sites on the proU promoter region. However, both proteins were unable to undergo the polymerization step on the DNA.

The same experiments were performed with two proteins modified in their carboxyl-terminal domain, namely the H-NS I119T and the Y97C. Both proteins, notably Y97C, previously exhibited a poor specificity of recognition of the proU promoter sequence among the pBR322 restriction fragments (see above). Upon DNase I attack and in the presence of increasing amounts of protein, footprints at discrete sites may still be observed, but they occurred at much higher protein concentrations than with the wild-type protein, their appearance being almost coincident with the complete protection of the fragment (Fig. 3, I (lanes j–m) and III).

The two proteins modified in their COOH-terminal domain were therefore still able to cover the proU DNA fragment, but their affinity for specific sequences was hampered. In terms of the proposed model, we suggest that the nucleation step required for the build up of a specific oligomer on the DNA is now weakened and is efficiently competed by random initiations of polymerization on the proU DNA fragment.

Oligomerization State of the Various H-NS Proteins in Solution

Analytical Ultracentrifugation

Equilibrium sedimentation experiments were performed with the amino-terminal domain of the H-NS protein (H-NS Δ64) (Fig. 4a). The molecular mass determined from these experiments was 16.95 kDa. The molecular mass of each monomer, as determined by mass spectrometry, is 8240 kDa (data not shown). Under our experimental conditions, the H-NS Δ64 behaves therefore as a dimer in solution. Sedimentation velocity experiments, performed under the same experimental conditions, indeed showed the presence of a dominant species, identified here as a dimer, sedimenting at $s_{20,w} = 1.9$ S (Fig. 4b).

For the wild-type H-NS, as well as for the H-NS L26P, equilibrium sedimentation experiments could not be performed, since the protein aggregated as a function of time (data not shown). Fig. 4c displays the distribution of sedimentation coefficients as obtained from sedimentation velocity experiments performed on the wild-type protein at a total concentration in soluble material of 50 μM. Two major species were found to be present, sedimenting at 3.9 S (76%) and at 1.8 S (24%), respectively.

When this experiment was performed with the same total concentration in the H-NS L26P, about 50% of the protein was lost due to aggregation, indicating the poor solubility of this modified H-NS under these experimental conditions. Fig. 4d presents a sedimentation velocity experiment performed at a total concentration in soluble material of 25 μM. As for the wild-type material, two major components were observed at 1.8 S and in the 3.8–4.5 S range. However, this time, the major component was clearly sedimenting at 1.8 S, a position matching with the slowest migrating component in the pattern observed with H-NS wild type.

At the protein concentration used in these experiments, it is clearly established that the equilibrium concentration of wild-type H-NS monomer is extremely small (22, 37). The 1.8 S component of H-NS in velocity experiments is thus at least a dimer. The presence of a species sedimenting at 1.8 S then reveals abnormal sedimentation behavior for the wild-type pro-
tein, since this value is smaller than the one obtained for the H9004 Δ64 dimer. It must be concluded, in good agreement with recent NMR experiments (38), that the shape of the H-NS wild type is far from spherical. Indeed, the NMR signal of the whole H-NS protein was found to be strikingly similar to that of its amino-terminal domain, suggesting that the latter is moving freely in the whole protein. The aggregation state of the more slowly sedimenting component cannot be determined with accuracy, but the observation of this species clearly indicates that wild-type H-NS forms oligomeric forms higher than dimers in solution. For the H-NS L26P, the component sedimenting as a dimer represents the dominant protein form at 25°C protein concentration.

Protein Cross-linking

Another way to compare the oligomerization state of the H-NS variants, and possibly to assess the association regions of the proteins that are mainly affected by the mutation, is to use a chemical cross-linking approach. A two-step chemical cross-linking reaction with the reactants EDC and NHS was performed (39). The cross-linking reaction induces formation of a peptide bond between an acidic amino acid (glutamate or aspartate) with a primary amine (lysine) when these two residues are in close contact. The efficiency of cross-linking depends mainly on the distance between the two reactive partners but also on their steric accessibility and on the ionic and hydrophilic environments of the relevant partners.

Cross-linking experiments were performed at 100 μM protein with 40 mM EDC, 10 mM NHS, as a function of reaction time. Fig. 5A shows experiments performed with the H-NS Δ64. As expected, a single product appeared on SDS-PAGE after cross-linking, at a position expected for a cross-linked dimer. The total yield of the reaction was 60 ± 10% after 45 min of incubation. When the wild-type H-NS was cross-linked under the same conditions, the total yield was roughly the same, but the reaction was faster. After 2 min, a cross-linked product appeared, at a position expected for a dimer (31 kDa). At later times, despite reagent inactivation higher oligomeric cross-linked products appeared as a smear in the gel. A faint band that is likely to correspond to the trimer could also be detected. We assume that this trimer results from secondary cross-links between a cross-linked dimer and one of the monomeric units of a non-cross-linked dimer. Since the interface involved in this secondary cross-link is unlikely to be the same as that comprising the dimer interface, the relative cross-linking efficiency may not be identical. Upon the appearance of the higher oligomeric forms, the percentage of cross-linked dimer remained roughly constant (Fig. 5B). Similar phenomena occurred with the protein modified in its carboxyl-terminal H-NS Y97C (Fig. 5C). By contrast, only cross-linked dimers were observed for the proteins modified in their amino-terminal domains, H-NS E53G/T55P and H-NS L26P, even after a longer cross-linking reaction time (45 min) (Fig. 5D). The absence of a significant amount of cross-linked oligomers larger than dimers with these proteins (as with the Δ64 polypeptide) suggests that in the two cases, protein-protein contacts are missing in one of the two interfaces, although this result as a whole may be considered as a control indicating that, under our experimental conditions, no nonspecific cross-linking occurred.

To quantitatively compare the cross-linking ability of the various proteins, we followed the kinetics of disappearance of the monomer species on the gel and monitored the overall yield of the reaction. These experiments were performed with the wild-type protein, the H-NS Δ64, and the H-NS L26P. The E53G/T55P protein exhibited a more drastic loss of cross-linking reactivity (data not shown) and was not used in the course of this experiment. The reaction conditions were first optimized by performing chemical cross-linking with various EDC and

![Fig. 3: DNase I footprinting by wild type and modified H-NS on the proU promoter at 10°C. I, DNase I footprints on the coding strand (lanes f, i, and n, 0 mM H-NS). Lanes a–e, H-NS wild type at 0.02, 0.05, 0.15, 0.5, or 1.0 μM. Lanes g and h, 0.05 and 1 μM H-NS L26P. Lanes j–m, 0.05, 0.1, 0.5, or 1.0 μM H-NS Y97C. II, lanes a–h, H-NS E53G/T55P at 0, 0, 0.1, 0.5, 1.0, 2.5, 0, and 0 μM, respectively. III, lanes a–i, H-NS I119T at 0, 0, 0.05, 0.1, 0.5, 1.0, 2.0, 0, and 0 μM, respectively.](image-url)
H-NS. The H-NS Δ64 domain appears to be a dimer (denoted below as core dimer) in the protein concentration range tested. The corresponding interface reacted poorly to the cross-linking reagent (low value of $k_{obs}$). The L26P protein also behaved essentially as a dimer in the cross-linking assays, although some higher oligomers were revealed by sedimentation velocity. For the wild-type protein, oligomerization states higher than the dimer have to be taken into account. Dimeric products could arise by two competing processes: cross-linking of the core dimer, occurring at the same rate as with the L26P protein (rate constant $k_1$), or cross-linking at the second interface, which probably leads to polymerization, a process which is highly concentration-dependent on the initial concentration of protein (rate constant $k_2$). The cross-linking experiments are well accounted by the simple equation,

$$k_{obs} = k_1 + k_2 p(c)$$  \hspace{1cm} (Eq. 3)

where $(p(c))$ represents the probability to form the second interface as a function of the total protein concentration.

**DISCUSSION**

In this study, we have performed biochemical studies on wild-type H-NS and on a series of dominant negative H-NS mutants. We wanted to clarify the properties altered by these various mutations and to correlate them with previous functional studies in order to determine which protein domain is implied in a given function, and thus determine which step occurring during the formation of a nucleoprotein structure on a given DNA fragment is altered.

From the electrophoretic mobility shift assay experiments (Fig. 1), three classes of dominant negative mutants may be distinguished: (i) mutants modified in the amino-terminal domain are still able to recognize the same specific DNA targets as the wild-type protein, L26P, and E53G/T55P; (ii) mutants
modified in the carboxyl terminal domain (Y97C, P116S, and I119T) can bind to DNA but lose the ability to recognize specifically those DNA sequences preferentially bound by the WT protein, proU and bla; and (iii) one mutant (the NH2-terminal Δ64 peptide) completely loses the ability to bind DNA. Different functions may therefore be clearly attributed to the two main
domains of the H-NS monomer. All of these results are in fair agreement with previous work reported in Ref. 25.

In *E. coli* H-NS, the basic unit (the core) is a dimer. Oligomerization of this core occurs in solution as well as when the protein is stably bound to DNA. All of the various NH$_2$-terminal domains of H-NS-like proteins examined to date are reported to be dimeric$^3$ with the notable exception of the Δ64 peptide from *S. typhimurium* (38). *E. coli* Δ64 H-NS analyzed here by equilibrium sedimentation behaves as a dimer. The time course of formation of cross-linked products, after reaction with EDC and N-HS, strongly suggests that this dimeric core is conserved in the integral protein. There is a marked decrease in the cross-linking efficiency after formation of the first dimeric product, a feature that is inconsistent with a rearrangement leading to a trimeric core. In fact, the overall analysis of the reactivity of the protein toward the present cross-linking reagent fully agrees with the existence of two subunit interfaces in *E. coli* H-NS, one that holds tightly the dimer and another that allows polymerization of the core. The biphasic nature of the dependence of the rate constant for monomer disappearance when the total protein concentration is increased is fairly accounted for by the lower reactivity of the polymerization interface. Indeed, the increase in the corresponding rate constant occurs in a protein concentration range where the fraction of H-NS oligomers higher than the dimer becomes significant in solution (22, 37).

Previous genetic and biochemical studies have established that the 64 NH$_2$-terminal domain of the protein is sufficient for the formation of the core (23, 26). A further extension to positions 90–95 is required for the truncated protein to display a significant amount of higher order multimers (25, 38). A naive interpretation of these results will locate the dimeric and the oligomerization interfaces in peptides 1–64 and 65–90, respectively. In agreement with this first-order approximation, we have observed that a mutant of the COOH-terminal region does not affect oligomerization in solution. Also, the removal of the

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3 M. Kochoyan, personal communication.
the DNA fragment at a late stage of its binding to DNA, forming during this step a precisely defined nucleoprotein structure capable of inhibiting transcription (7). This conclusion is in good agreement with recent microscopic studies, which showed the formation of filamentous structures when visualizing H-NS-DNA complexes (41, 42). These filaments are constituted by large tracts with two regions of double-stranded DNA held close together (41). It was proposed, from their observation, that the binding of H-NS to DNA would occur through a nucleation step followed by a zipper-like propagation along the DNA (42). It is still unclear whether these images are relevant to the 372-bp fragment that was used in this work, but it is tempting to draw a parallel between the images obtained from microscopy and the footprint experiments. In this case, what we define as the polymerization step could correspond to the formation of a filament-like structure, and the propagation step in our model would represent the formation of an early nucleoprotein structure close to the curved region of DNA, necessary for the filament formation to occur. The appearance of discrete footprints (in our model of propagation following the nucleation step on the curved sequence) could correspond to the formation of a nucleoprotein complex. One way in which we are pursuing these studies is through the use of time-resolved footprinting, which will allow discrimination of specific intermediates.

Footprints obtained with the dominant negative mutants of H-NS modified in the amino-terminal domain (L26P and E53C/ T55P) show that only specific sites are protected at any protein concentration. The absence of the polymerization step is thus probably due to the same alteration in protein-protein interaction as that observed in solution, the weakening of the interaction interface. The dimer formed by these mutated proteins is thus the smallest unit that can recognize and bind specifically to DNA, and this structure is probably the one involved when the core protein recognizes its specific sites on DNA prior to extensive propagation. The formation of such a fiber by the single H-NS protein requires head to tail association of the same repeating protein motif, the core dimer. The dominant negative effect of these mutants implies disruption of this type of assembly, as already postulated by Williams et al. (23).

The Δ64 dimer has lost its capacity to bind to DNA. However, the Δ64 protein behaves as a clear dominant negative mutant, probably because its truncated monomer can interact with a wild-type H-NS and weaken the interaction with the DNA template as well as with neighboring bound H-NS dimers, during nucleoprotein assembly.

Footprinting experiments performed with the dominant negative mutants modified in the carboxyl terminus domain show that these mutant proteins are able to induce a general protection of the DNA without prior recognition of specific sites at NRE. Cross-linking experiments performed with these mutants show that they reach the same oligomerization state as the WT protein in vitro (Fig. 3D). These mutants thus retain their full ability to form protein-protein interactions. Changes in the carboxy-terminal domains probably induce destabilization of the hydrophobic core of the protein. It is indeed striking that most of the changes concern amino acids involved in the formation of this core, replacement residues being less hydrophobic (I119T and Y97C). This might induce an increase in the formation of this core, replacement residues being less hydrophobic than the WT protein (43). We suggest instead an explanation in line with that invoked to explain the loss of strong repression exerted by H-NS when a straight fragment was substituted for a curved one upstream at the gal promoters (7). The nucleoprotein structures formed in the absence of recognition of specific sites or in the absence of a curved sequence acting as a nucleation site are more labile and therefore inefficient for the control of transcription in vivo.

The same set of interacting protein surfaces seems therefore to operate during the formation of oligomers in solution and as binding proceeds on a DNA template. Significant global rearrangements of the protein could, however, take place during the transfer of H-NS oligomers from solution to DNA. In solution, oligomerization rarely extends beyond the tetramer (22, 37) in the 0.2 μM to 1 μM concentration range. During their analysis of wild-type H-NS self-assembly equilibria in solution, Ceschini et al. have found that a temperature increase favors tetramer formation (22). On the contrary, we have found that at proU, polymerization is strongly hampered when the temperature was raised by 27 °C. It is not unlikely that the H-NS tetramer might undergo a significant conformational change to participate in the postulated head to tail assembly on the DNA template (e.g. a conversion from a closed to an open mode of polymerization). The documented flexibility of the two-domain wild-type structure could favor such a rearrangement. Furthermore, it is reported that, in vivo at proU, a temperature increase leads also to an activation of the P1 promoter (32). Therefore, changes in the strength of protein-protein contacts as well as DNA conformational changes (postulated by Falconi et al. (9)) could contribute to the sharp regulation exerted by temperature on prokaryotic promoters controlled by H-NS.

Finally, our experiments address the issue of specific versus non-specific recognition of DNA sequences by H-NS. The binding specificity, observed by electrophoretic mobility shift assay, is correlated with the existence of discrete sites located within DNA fragments that are protected at low concentrations of wild-type proteins. How H-NS binds nonspecifically to DNA and what are the structural differences between nucleoprotein complexes built from the recognition of a specific DNA sequence and/or non-specific sequences are questions that must be addressed in order to obtain a further understanding of H-NS function. It is important to note that the existence of these two different binding modes could help in understanding the plurality of H-NS function. Specific binding, requiring a nucleation step and polymerization of H-NS over the promoter(s) regulated by the protein, is likely to be at the origin of the regulatory function of H-NS. Nonspecific DNA binding, due to the oligomerization of H-NS to any DNA strands, could still play a significant role in the compaction of the nucleoid exerted by H-NS.

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