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# The Nucleoid Protein H-NS Facilitates Chromosome DNA Replication in *Escherichia coli dnaA* Mutants

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**Growth inhibition of the *dnaA*(Cs) mutant, which overinitiates chromosome replication, was shown to be dependent upon the nucleoid protein H-NS. [$^3$H]thymine incorporation experiments indicated that the absence of H-NS inhibited overreplication by the *dnaA*(Cs) mutant. In addition, the temperature-sensitive phenotype of a *dnaA46* mutant was enhanced by disruption of H-NS. These observations suggest that H-NS directly or indirectly facilitates the initiation of chromosome replication.**

Prokaryotic histone-like proteins play important roles in various DNA transactions (6, 23, 27). In *Escherichia coli*, histone-like proteins such as HU, IHF, and FIS are known to participate in chromosome replication.

Binding of HU to DNA is largely nonspecific and causes bending of the DNA (10). IHF binds to DNA in a sequence-specific manner (4). The origin for chromosome replication, *oriC*, contains an IHF-binding site, and IHF binding to the site causes DNA bending (28). In in vitro replication systems of minichromosome (*oriC* plasmid) reconstituted with purified proteins, HU is necessary for the process of initiation of replication (5, 25, 34), and IHF can substitute for HU in these systems (12, 31). Mutant cells lacking both HU and IHF grow slowly and take on a filamentous shape (14). Chromosome replication in the double mutant is aberrant in that minichromosomes are not maintained (15).

FIS also seems to be stimulatory for chromosome replication (7, 9, 29). Although this protein is nonessential for cell growth, the FIS-lacking mutant is far less competent for transformation of minichromosome than is the wild-type strain. FIS binds to the *oriC* region in a sequence-specific manner and causes bending of the *oriC* DNA.

H-NS, another histone-like factor, is an abundant DNA-binding protein with about 20,000 molecules per cell (33). Like HU, IHF, and FIS, H-NS is nonessential for cell growth, yet simultaneous depletion of HU, IHF, and H-NS renders cells inviable (38), which means that functional similarity probably exists. On the other hand, overexpression of H-NS in vivo causes extreme condensation of the chromosome, and such being the case, this protein is likely to have a unique role in the nucleoid structure (32). In the mutant lacking H-NS protein, expression of >50 genes is altered, either increased or decreased (2, 37, 39). Furthermore, while this protein has a high affinity to DNA, basically in a sequence-independent manner (8), there is preferential binding to DNA with intrinsic curvature (26, 36). Although the *oriC* region is known to contain a curved site (22), and other histone-like factors are well characterized, the role of H-NS in chromosome replication is poorly understood.

In this study, to examine the role of H-NS in the initiation of chromosome replication, we used *dnaA* mutants that affect initiation activity of chromosome replication. DnaA protein, encoded by the *dnaA* gene, binds to the *oriC* region, causes local unwinding, and leads to initiation of synthesis of cDNA strands (23). We found that disruption of the *hns* gene encoding H-NS suppresses the growth defect and inhibits the excessive replication seen in the *dnaA*(Cs) mutant (3, 21). In addition, growth of a temperature-sensitive *dnaA* mutant at a semipermissive temperature was greatly inhibited by introduction of the *hns* mutation. We suggest that H-NS has an important role in processes of initiation of chromosome replication in vivo. This effect may derive from a direct interaction of H-NS with the *oriC* region or may be an indirect consequence with alterations in gene expression and nucleoid structure.

**Suppression of *dnaA*(Cs) by an *hns*-null mutation.** The growth of the *dnaA*(Cs) mutant is inhibited at 39°C or below and is accompanied by an overinitiation of chromosome replication. We asked whether *hns* mutation would suppress the growth defect of the *dnaA*(Cs) mutant (Table 1 and Fig. 1). The Δ*hns*::*neo* mutation was introduced into the *dnaA*(Cs) strain (NA001) by P1 transduction; transductants appeared at 42°C with a frequency similar to that seen when the parental *dnaA*$^+$ strain (KH5402-1) was the recipient (data not shown). The resultant double mutant, MK19, and NA001 were grown overnight in Luria-Bertani medium (30) supplemented with 50 μg of thymine per ml at 42°C, diluted, and plated on the Luria-Bertani agar plates supplemented similarly. Each plate was incubated at 30, 35, 37, or 42°C for 24 h. The *dnaA*(Cs) *hns* double mutant grew well even at the low temperatures that severely inhibited growth of the *dnaA*(Cs) mutant (Fig. 1). At 30°C, the suppression seemed to be partial since colonies of the double mutant were tiny.

**Inhibition of overreplication by the *hns* mutation.** Initiation of chromosome replication is absolutely dependent on con-

TABLE 1. Bacterial strains

| Strain[a] | Relevant genotype | Source and/or reference |
|---|---|---|
| CSH4100 | Δ*hns*::*neo* | T. Mizuno (37) |
| KH5402-1 | *thyA ilv* | 24 |
| KA413 | KH5402-1 *ilv*$^+$ *dnaA46* | 20 |
| KA441 | KH5402-1 *dnaA*(Cs) *tna*::Tn*10* | 19 |
| KA837 | KA413 Δ*hns*::*neo* | This work |
| NA001 | KA441 free of Tn*10* | 1 |
| MK19 | NA001 Δ*hns*::*neo* | This work |
| MK21 | KH5402-1 Δ*hns*::*neo* | This work |

[a] All strains are derivatives of *Escherichia coli* K-12.

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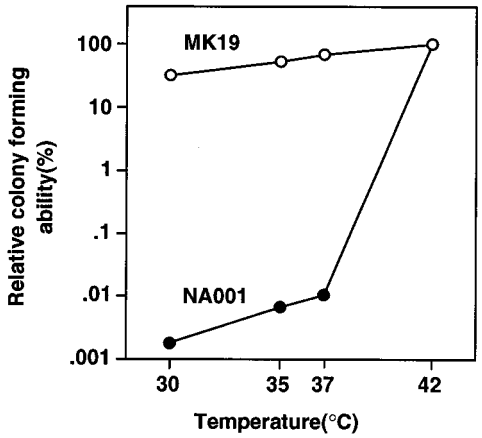


FIG. 1. Suppression of *dnaA*(Cs) cold sensitivity by *hns*-null mutation. Colonies formed at indicated temperatures were counted. Experiments were duplicated independently, and the mean is shown. Colonies of MK19 formed at 30°C were tiny. Symbols: ●, NA001 [*dnaA*(Cs)]; ○, MK19 [*dnaA*(Cs) Δ*hns*::*neo*].

comitant protein synthesis in the *dnaA*$^+$ strain (35). Conversely, in the *dnaA*(Cs) mutant, initiation occurs repeatedly in the presence of chloramphenicol (21), probably because the activity of DnaA(Cs) protein is stable for a much longer time than is that of the wild-type protein (16–19). We then asked whether the lack of H-NS would affect the overreplication seen in the *dnaA*(Cs) mutant (Fig. 2). Chromosome DNA in strains NA001 [*dnaA*(Cs)] and MK19 [*dnaA*(Cs) *hns*] was wholly labelled by overnight incubation at 42°C, in tryptone medium (21) containing 50 μg (3 μCi) of [*methyl*-$^3$H]thymine per ml. The resultant cultures were diluted 100-fold in the same medium as above and were grown exponentially at 42°C. When the $A_{660}$ of the cultures reached 0.1, chloramphenicol (200 μg/ml) was added and the cultures were immediately shifted to

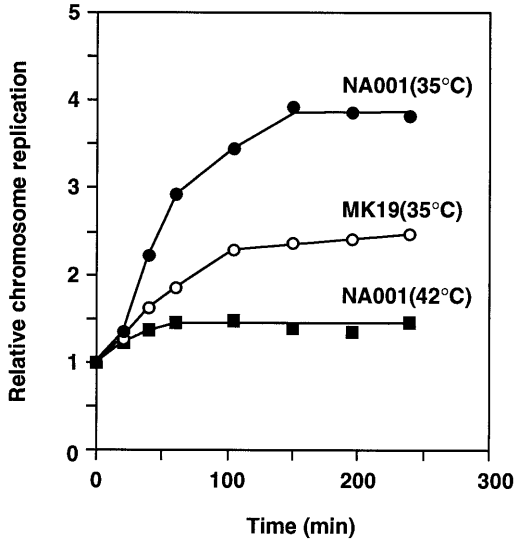


FIG. 2. Inhibition of overreplication of chromosome. Chromosome replication was measured by incorporation of [*methyl*-$^3$H]thymine into acid-insoluble materials. Samples were harvested at the indicated time after the addition of chloramphenicol. Radioactivities incorporated are indicated as a relative value. Each value is the mean of two measurements. An independent, second experiment yielded similar results. Symbols: ●, NA001 [*dnaA*(Cs)] at 35°C; ○, MK19 [*dnaA*(Cs) Δ*hns*::*neo*] at 35°C; ■, NA001 [*dnaA*(Cs)] at 42°C.

TABLE 2. Growth of the *dnaA46 hns* mutant and the parental strains[a]

| Strain | Relevant genotype | | Doubling time at 30°C (min)[b] | CFU/ml at 30°C[c] | Colony-forming ability[c] | |
|---|---|---|---|---|---|---|
| | *dnaA* | *hns* | | | 37°C/30°C | 42°C/30°C |
| KH5402-1 | + | + | 57 | $6.0 \times 10^9$ | 0.95 | 1.1 |
| MK21 | + | Δ | 60 | $2.1 \times 10^9$ | 1.2 | 1.0 |
| KA413 | *46* | + | 57 | $4.2 \times 10^9$ | 1.1 | $2.1 \times 10^{-7}$ |
| KA837 | *46* | Δ | 75 | $1.4 \times 10^9$ | $2.6 \times 10^{-5}$[d] | $<3.6 \times 10^{-8}$ |

[a] Luria-Bertani medium supplemented with 50 μg of thymine per ml was used.
[b] Growth was measured at $A_{660}$.
[c] Cells grown overnight at 30°C were used.
[d] Colonies formed at 37°C were tiny pinpoints.

35°C or subsequently kept at 42°C. Samples (200 μl) were withdrawn at various intervals, incubated at 0°C in trichloroacetic acid (7%), and passed through GF/C filters, and radioactivities in the insoluble materials were measured (20). As expected, chromosome replication in the *dnaA*(Cs) *hns* double mutant was significantly inhibited (Fig. 2). At 37°C, consistent results were obtained (data not shown). These data indicate that occurrence of replicational initiation or synthesis of DNA chain is inefficient in the double mutant, and thus H-NS seems to have a role, direct or indirect, in facilitating chromosome replication.

**Growth inhibition of a temperature-sensitive *dnaA* mutant.** We further examined the role of H-NS for initiation with a *dnaA46* mutant. This *dnaA46* mutant grows only at 38°C or below, and activity of the DnaA46 protein is temperature sensitive (11). At 35°C, activity of this protein for minichromosome replication in vitro is largely decreased, compared with that at 30°C, and even at 30°C, the activity is only about 25%, compared with that of the wild-type protein.

The *hns* mutation was introduced into a *dnaA46* mutant by P1 transduction, and transductants were obtained at 30°C. The transduction efficiency was similar to that seen when the parental *dnaA*$^+$ strain was the recipient (data not shown). At 30°C, a slight reduction in growth rate was observed for the *dnaA46 hns* mutant, compared with that of the parental strains (Table 2). Using cells grown at 30°C, we determined CFU by incubation at 30, 37, and 42°C, for 24 h. The double mutant showed severe growth inhibition at 37°C whereas the *dnaA46* mutant and the parental strains grew well at this temperature (Table 2). Thus, H-NS is apparently a component required to enhance the initiation of chromosome replication.

H-NS may modulate the expression of genes to facilitate the process of the initiation. Alternatively, conformational change in the nucleoid structure by H-NS may be related to the initiation of chromosome replication. Although we did not observe the effect of H-NS on the initiation in the *dnaA*$^+$ background, Kaidow et al. (13) recently found that, in a *dnaA*$^+$ Δ*hns*::*neo* mutant, the ploidy of chromosome per cell decreases, and anucleate cells are produced. The inhibited replication of the chromosome may perhaps relate to this occurrence.

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