Protective Role for H-NS Protein in IS601 Transposition

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The transposase (InsAB') of the insertion element IS601 can create breaks in DNA that lead to induction of the SOS response. We have used the SOS response to InsAB' to screen for host mutations that affect InsAB' function and thus point to host functions that contribute to the IS601 transposition mechanism. Mutations in the hns gene, which codes for a DNA binding protein with wide-ranging effects on gene expression, abolish the InsAB'-induced SOS response. They also reduce transposition, whether by simple insertion or cointegrate formation, at least 100-fold compared with the frequency seen in hns' cells. Examination of protein profiles revealed that in an hns-null mutant, InsAB' is undetectable under conditions where it constitutes the most abundant protein in hns' cells. Likewise, brief labeling of the hns cells with [35S]methionine revealed very small amounts of InsAB', and this was undetectable after a short chase. Transcription from the promoters used to express insAB' was essentially unaltered in hns cells, as was the level of insAB' mRNA. A mutation in lon, but not in fisH or clpP, restored InsAB' synthesis in the hns strain, and a mutation in ssaA partially restored it, implying that the absence of H-NS leads to a problem in completing translation of insAB' mRNA and/or degradation of nascent InsAB' protein.

Active transposable elements appear to exhibit a high degree of autonomy. Typically, the transposase encoded by the element interacts specifically with the element’s ends, locates a target sequence, and executes the cleavage and ligation reactions required to insert the element into the new site. Nevertheless, host functions can be intimately involved, most obviously in the case of the Mu prophage, where replication and transposition of the Mu prophage are essential for the initiation of the transposition pathway (11, 36). To identify host functions that regulate IS601 transposition, we used the SOS response induced by InsAB', as a screen for the inhibitory effects of mutations in candidate genes. Mutant alleles of most genes, including hmsD (IHF subunit) and fis, did not affect the SOS response to InsAB', but a Tn10 insertion in the hns gene reduced it markedly (see Results). This paper is a report of our attempts to find out why.

H-NS is a small (15-kDa) abundant (~20,000 molecules per cell) protein that plays a major role in compaction of the E. coli chromosome (43, 44). In binding to DNA, it shows a strong preference for curved regions (48). It modulates the transcription of many genes, usually as a repressor (1). In view of the precedent cited above, we expected that H-NS would affect IS601 transposition by directly modulating the transposition pathway. Our results show, however, that it intervenes at another point in IS601 transposition.

MATERIALS AND METHODS

Bacterial strains and plasmids The strains used were derivatives of E. coli K-12 and were essentially as described previously, with genotypes (25). The transformation recipient for plasmid constructions and the host for InsAB' production experiments was MC1061; the SOS reporter strain was BR293; the donor strain for transformation assays was C600 Δ(mfd-recA)306::Tn10 carrying the conjugative transposon target plasmid, pOX38::dTn10 cat (Cm'), or when

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TABLE 1. Plasmids used and constructed

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Relevant characteristicsa</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAPT1</td>
<td>orFusA kan, lac:z+1857</td>
<td>D. Zerbib</td>
</tr>
<tr>
<td>pMET37</td>
<td>orFusA::bla plasmid insAB* B'</td>
<td>D. Zerbib</td>
</tr>
<tr>
<td>pMET12</td>
<td>As pMET37 but insAB*</td>
<td>H.11032</td>
</tr>
<tr>
<td>pMET37</td>
<td>orFusA::bla lac::insAB* (GA2G3AC) lacP*</td>
<td>D. Zerbib</td>
</tr>
<tr>
<td>pMET35</td>
<td>As pMET37 but InsAB*</td>
<td>D. Zerbib</td>
</tr>
<tr>
<td>pTemp1.1</td>
<td>orFusA::bla lac+Z</td>
<td>D. Zerbib</td>
</tr>
<tr>
<td>pDAG98</td>
<td>As pMET37 but insAB* (codon 127: lacZYA)</td>
<td>D. Zerbib</td>
</tr>
<tr>
<td>pDAG99</td>
<td>As pMET37 but insAB* (codon 220: lacZYA)</td>
<td>This work</td>
</tr>
<tr>
<td>pDAG92</td>
<td>orFusA::bla araC araBADp insA::lacP</td>
<td>This work</td>
</tr>
<tr>
<td>pMEM8</td>
<td>orFusA::bla lac+spec</td>
<td>30</td>
</tr>
<tr>
<td>pCST200</td>
<td>orFusA::bla araC araBADp insAB* B'</td>
<td>37</td>
</tr>
<tr>
<td>pCST400</td>
<td>As pCST200 but insAB* (GA2G3AC)</td>
<td>37</td>
</tr>
</tbody>
</table>

a InsAB* is the artificial transposable element composed of two IRL ends flanking a spectinomycin resistance gene (aadA) and the transcription terminator of T4 gene 32 (31).

The medium for routine growth was Luria Broth (LB) broth supplemented with 15% agar for solid medium and, as appropriate, with the antibiotics ampicillin (100 μg/ml), spectinomycin (100 μg/ml), kanamycin (30 μg/ml), chloramphenicol (20 μg/ml), tetracycline (12.5 μg/ml), and gentamicin (2.5 μg/ml). Cultures were grown at 37°C except otherwise noted. The medium for Leu- frames was 2% glucose (0.2% lysine, 0.5% Difco methionine assay medium). The medium for selection of Lon (A7C) was made from the immediate ancestor of pMET37 by deletion of most of the IS1 transposable element composed of two IRL ends (isomeron [Ωlon]) (31) present in both pMET37 and pMET12.

Media and growth conditions. The medium for routine growth was Luria Broth (LB) broth supplemented with 15% agar for solid medium and, as appropriate, with the antibiotics ampicillin (100 μg/ml), spectinomycin (100 μg/ml), kanamycin (30 μg/ml), chloramphenicol (20 μg/ml), tetracycline (12.5 μg/ml), and gentamicin (2.5 μg/ml). Cultures were grown at 37°C except otherwise noted. The medium for Leu- frames was 2% glucose (0.2% lysine, 0.5% Difco methionine assay medium). The medium for selection of Lon (A7C) was made from the immediate ancestor of pMET37 by deletion of most of the IS1 transposable element composed of two IRL ends (isomeron [Ωlon]) (31) present in both pMET37 and pMET12.

RESULTS

Loss of SOS response to InsAB in hns mutants. To produce InsAB* at levels that allow the SOS response to be readily detected, we used two systems, both of them employing insAB sequences in which single-basepair insertions in the frameshift motif A6C had fused the insA and insB sequences in phase, thus relieving InsAB* production from dependence on inefficient frameshifting. The first system involves two compatible plasmids, one carrying the fused-frame (A7C) insAB* sequence under the control of the λ P1 promoter (pMET12) and the other carrying the gene for the temperature-sensitive repressor, CI857 (pAPT1); InsAB* synthesis is induced by raising the growth temperature to 39°C. In the second system, a lacP-controlled fused-frame (GA2G3AC) insAB* and the lacP* repressor gene are carried on a single plasmid (pMET37). An artificial IS1 element (isomeron [Ωlon]) (31) present in both InsAB* producer plasmids to provide ends for cleavage by transposase.
These plasmids were introduced into an SOS reporter strain that carries a *km*434 prophage with its P₁ promoter fused to the *lacZ* gene; cleavage of the 434 repressor following SOS induction results in β-galactosidase synthesis, which is detected on indicator medium or measured in samples of liquid cultures (9). Induction of the λ P₁-controlled insAB⁺ (A7C) gene in this strain raised β-galactosidase specific activity above the background level of 149 U, to 859 U (Table 1). Most of this increase resulted from the presence of IS₁ ends on the plasmid, since in their absence β-galactosidase rose only modestly, to 256 U (presumably through action on chromosomal IS₁ ends). Derivatives of the reporter strain carrying hns-::Tn10 and Δhns::kan alleles were constructed. The SOS response to InsAB⁺ induction in the hns-::Tn10 mutant carrying pMET12 was much lower (264 U) than in the hns⁺ reporter. The difference was even more marked in the experiment, shown in Table 2, which employed the Δhns::kan derivative. Here, full induction of *lac* promoter activity with IPTG and an insA-insB joint sequence on which reverse frameshifting does not occur (GA2GAC3) (11) led to higher levels of InsAB⁺ and an increased SOS response in hns⁺ cells. Residual synthesis in the Δhns mutant, however, was as low as in the hns-::Tn10 mutant.

The P₁ and *lac* promoters were just as active in the Δhns strain as in the wild type (Fig. 1a and b), and detection of SOS induction was just as responsive (Fig. 1d). The results imply that the absence of H-NS retards IS₁ transposition. We next tested this suggestion directly.

**IS₁ transposition in hns mutants.** Transposition frequency was measured by the mating-out assay of Chandler and Galas (5). A ΔrecA derivative of strain C600 carrying the F', pOX38-Cm, was transformed with the transposon donor plasmid pMET37, which carries *bla* (Ap'), *lacp*:insAB⁺ (GA2GA3C), and the repres sor gene, lacP', as well an artificial IS1 element (Δhns) composed of two IRLs flanking the *aadA* (spectinomycin resistance) gene. Transposition to the F' was measured as the fraction of F' (Cm') exconjugant recipients that were also Ap' Sp' (cointegrates) or Sp' (cointegrates plus simple insertions). The first two lines of Table 3 show the main results. Transposition in the Δhns strain was ∼3,000-fold lower than in the wild type, scarcely above background levels. When the donor cells had been grown in the presence of IPTG to induce InsAB⁺ production (Table 3, third and fourth lines), transposition in the wild-type was no higher than in the absence of IPTG; presumably, escape synthesis from *lacp* provided enough InsAB⁺ for the maximum rate of transposition in this system. In the Δhns strain, however, IPTG induction increased transposition ∼35-fold. The hns mutation did not significantly affect the frequency of cointegrates relative to direct insertion events.

To examine the possibility that the H-NS effect we had seen might be an artifact resulting from very high levels of InsAB⁺, we measured transposition from Ap⁺ donor plasmids which carried a natural IS1 and Ωon-spc (pMP3) or Ωon-spc alone (pMET8); transposition from the latter plasmid depends on InsAB⁺ produced from chromosomal IS₁ copies and can be taken as the background for the assay. Transposition from pMP3 in the Δhns strain was again close to background levels, 300-fold lower than in the hns⁺ strain. This result indicates that the disparity between transposition rates in hns⁺ and Δhns strains reflects an authentic involvement of H-NS in IS₁ transposition. The frequencies of Ap⁺ Sp⁺ exconjugants from matings with the pMET8 strain were very low for both strains. The elevated levels of apparent cointegrates led us to suspect that a significant fraction of the Sp⁺ F’ s were formed by recombination events other than transposition, as reported previously (23).

The observation that leaky synthesis from *lacp* allowed maximal rates of transposition in the wild type whereas induction of *lacp* led to higher rates in the Δhns strain suggested that in

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**TABLE 2. Effects of hns mutations on induction of the SOS response by IS₁ transposase**

<table>
<thead>
<tr>
<th>Promoter and gene</th>
<th>hns</th>
<th>Presence of:</th>
<th>β-galactosidase</th>
<th>sp act</th>
<th>(Miller units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P₁::insAB⁺ (A7C) at 39°C</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>149</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1,900</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ::kan</td>
<td>+</td>
<td>−</td>
<td>149</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ::kan</td>
<td>+</td>
<td>+</td>
<td>240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lacp::insAB⁺ (GA2GA3C) + IPTG</td>
<td>+</td>
<td>−</td>
<td>177</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>1,900</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**FIG. 1. Activities of promoters in hns mutant strains.** Cultures of isogenic hns⁺ (open symbols) and hns (solid symbols) strains growing exponentially in LB medium were shifted to inducing conditions (0 generations) and sampled at intervals for assay of β-galactosidase. (a) BR293 strains carrying pAP71 (C1857) and pTempl.1 (P₁::lacZ). (b) MC1061 strains carrying lacP' and lacp::insAB⁺::lacZ fusions on pDAG98 (circles) or pDAG99 (squares). (c) MC1061 strains carrying araC and araBADp::insA::lacZ on pDAG92. (d) BR293 strains (Δhns⁺; •, hns; •, hns::Tn10) carrying P₁::434::lacZ (SOS reporter fusion) at attI incubated with or without (−MC) 20 ng of mitomycin C/ml. The culture doubling times (±2 min) in LB at 37°C for hns⁺ and Δhns strains were 30 and 51 min (BR293) and 26 and 58 min (MC1061), respectively. The cultures entered stationary phase after −4 generations (a and b) or 4 h (d), ara, arabinose.

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TABLE 3. Effects of Δhns allele on IS1 transposition frequency

<table>
<thead>
<tr>
<th>Transposon donor plasmid</th>
<th>hns</th>
<th>IPTG (1 mM)*</th>
<th>Transposition frequency (± SD) (Spc/Cam')</th>
<th>n</th>
<th>Cointegrate fraction (Amp'/Spc')</th>
<th>Δhns/hns γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>pMET37 (lacz::insAB')</td>
<td>+</td>
<td>-</td>
<td>1.4 × 10^{-3} (±0.5)</td>
<td>3</td>
<td>0.62</td>
<td>0.00033</td>
</tr>
<tr>
<td></td>
<td>Δ</td>
<td>-</td>
<td>4.6 × 10^{-3} (±3.0)</td>
<td>3</td>
<td>0.60</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>1.2 × 10^{-3} (±0.2)</td>
<td>3</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Δ</td>
<td>+</td>
<td>1.6 × 10^{-5} (±0.7)</td>
<td>3</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>pMP3 (wild-type IS1)</td>
<td>+</td>
<td>-</td>
<td>1.1 × 10^{-4} (±0.3)</td>
<td>2</td>
<td>0.12</td>
<td>0.0032</td>
</tr>
<tr>
<td></td>
<td>Δ</td>
<td>-</td>
<td>3.5 × 10^{-1} (±1.4)</td>
<td>4</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>pMET8 (no insAB')</td>
<td>+</td>
<td>-</td>
<td>2.7 × 10^{-7} (±0.1)</td>
<td>2</td>
<td>0.93</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Δ</td>
<td>-</td>
<td>1.5 × 10^{-7} (±0.1)</td>
<td>4</td>
<td>0.95</td>
<td></td>
</tr>
</tbody>
</table>

*+, present; –, absent.

the latter strain low transposition rates might result from limited quantities of InsAB’. We next investigated InsAB’ levels.

InsAB’ production in Δhns cells. Cultures of strain DLT288 (Δhns) carrying pCST400, in which the insAB’ (GA2GAG3C) sequence is controlled by the ara promoter, and of the equivalent hns’ strain (DLT286) were treated with arabinose, and samples were taken for analysis of proteins by SDS-PAGE. A Coomassie blue-stained gel is shown in Fig. 2. Extracts of hns’ cells induced with arabinose (lane 4) or those of induced cells carrying the wild-type insAB’ (A6C). The 27-kDa species corresponds to the predicted size of InsAB’, 26.6 kDa. No band of this size was detected in Δhns samples, even those taken from cultures grown in the presence of arabinose for 2 h (lane 8). The araBADp promoter, like lacp and P_L, was just as active in Δhns cells as in hns’ cells (Fig. 1c). We conclude that in hns mutant cells, either the synthesis or the stability of InsAB’ is much reduced.

To determine the rate of InsAB’ degradation in each strain, we performed a pulse-chase experiment. Arabinose was added to log-phase cultures of DLT286 and -288, as described above, to induce insAB’ transcription. Nascent protein was labeled by the addition of [35S]methionine, and labeling was terminated 1 min later by the addition of excess nonradioactive methionine. Samples were withdrawn at intervals for assay of 35S-labeled InsAB’ protein by SDS-PAGE and radioautography. We were obliged to observe InsAB’ against a background of labeled host polypeptides, because qualitative immunoprecipitation of InsAB’, using antibodies raised against InsA protein, was not successful. Nevertheless, the radioautograph shown in Fig. 3a reveals that a prominent band of 35S-InsAB’ is present in the sample from pulse-labeled hns’ cells and that this declines upon subsequent incubation. In contrast, an InsAB’ band in the pulse-labeled sample from Δhns cells is barely detectable and was not visible at all in chased samples. The contrast between the amounts of labeled InsAB’ in the two strains is more readily seen on the radioautograph of a gel in which equivalent samples from hns’ and Δhns cells were run side by side (Fig. 3b). We estimate the half-life of InsAB’ in hns’ cells to be 13 min (Fig. 3c); there was too little signal above background to allow calculation of the half-life in Δhns cells.

Without a measurement of InsAB’ stability in Δhns cells, we cannot eliminate the possibility that H-NS normally intervenes in the synthesis of the transposase. We next examined the various steps of InsAB’ synthesis.

InsAB’ mRNA synthesis in Δhns cells. While the promoters used for expression of insAB’::lacZ fusions are just as active in hns mutants as in hns’ cells (Fig. 1), native insAB’ mRNA might be more sensitive to degradation in the mutants. This possibility was tested by Northern blot analysis of total RNA extracted from cultures of the same strains as those employed to test InsAB’ protein levels. Figure 4 shows the amounts of a labeled probe consisting of the entire IS1 sequence that hybridized with total RNA extracted from log-phase cultures of strains carrying IPTG-inducible and arabinose-inducible insAB’ genes. The amounts of insAB’ mRNA in induced Δhns cells were comparable to, or even higher than, those in the equivalent hns’ cells. Prentki et al. (32) reported the presence...
of a rho-dependent terminator in IS1; a higher efficiency of this terminator in hns mutants might reduce production of full-length mRNA. However, slot blot hybridization using a probe consisting of only the 3’/H11032 nucleotides of insB revealed comparable amounts of IS1-specific RNA in the hns/H11001 and hns/H9004 total-RNA preparations (data not shown). Absence of H-NS, therefore, does not interfere with InsAB protein production at the level of transcription or messenger stability.

InsAB’ mRNA translation in Δhns cells. Yamashino et al. (49) reported that in an hns null mutant, production of the RpoS sigma factor was ~15-fold higher from a given amount of mRNA than in the hns+ counterpart, while the rate of degradation of the RpoS protein was at 10-fold lower. These observations provide a precedent for the involvement of H-NS in both translation efficiency and protein stability. The data in Fig. 1b indicate, however, that translation of the insAB’ mRNA was not significantly affected by the Δhns mutation. In plasmids pDAG98 and -99, the N-terminal 126 and 205 codons, respectively, of the 232-codon insAB’ reading frame are fused to the 5’ end of lacZ. If there is a problem with translation of insAB’ mRNA, it must occur during reading of the last 27 codons or, conceivably, be suppressed as a result of the lacZ mRNA extension.

Mutation in lon suppresses InsAB’ deficiency in Δhns cells. We examined the abilities of mutant alleles of known proteolysis-related genes to restore InsAB’ synthesis in the Δhns strain. Figure 5a shows a Coomassie blue-stained SDS-polyacrylamide gel on which the proteins of lon and ssrA mutant derivatives of strains DLT288 (Δhns) have been resolved. A strong InsAB’ band is present in the extract of the lon mutant, and this band can also be detected in the ssrA mutant sample. No restoration of InsAB’ synthesis (or of SOS response) was seen upon introduction of ftsH, clpP, or clpX mutations into Δhns::Tn10 strains (data not shown). These results suggest that the InsAB’ protein is subject to specific degradation by Lon protease and the C-terminal proteolysis-marking mechanism governed by ssrA.

Extragenic suppressors of slow growth arise frequently in Δhns strains (20). It was therefore important to test whether the observed restoration of InsAB’ synthesis in the lon::Tn10 derivative was indeed due to the allele introduced and not to overgrowth by mutants carrying unknown suppressors. The lon+ allele was substituted for the mutant allele in the Δhns lon::Tn10 strain by P1 transduction, using selection for resistance to methyl methane sulfonate. Four transductants carrying pCST400 were tested for InsAB’ production following the addition of arabinose. All showed the absence of InsAB’ characteristic of the original Δhns strain (Fig. 5b).

To determine whether restoration of InsAB’ synthesis by the lon mutation is accompanied by the return of InsAB’ function,
transposition frequency in the Δhns lon::Tn10 strain was measured, using the mating-out system described above. The transposition frequency in the Δhns strain relative to that in hns+ did increase as a consequence of the introduction of the lon mutation (Table 4), though not to the hns+ level. However, inspection of the transposition frequency column reveals that the apparent shortfall results from a significant stimulation of transposition frequency that the lon mutation also causes in the hns+ strain. Transposition frequencies in the Δhns lon::Tn10 strain were actually comparable to those in the wild type (hns+ lon+): 0.23 × 10^-3 (compared to 0.73 × 10^-3) without IPTG induction and 2.6 × 10^-3 (compared to 2.1 × 10^-3) with induction. It is nevertheless possible that other functions affected by H-NS or Lon protease prevent transposition from reaching the very high level observed in the hns+ lon::Tn10 mutant.

**DISCUSSION**

We have shown that in hns mutants, the inability of ISI transposase to induce the SOS response or to stimulate its element to transpose at high rates results primarily from the cell’s failure to accumulate it in sufficient quantities. The transposase deficit appears to be created at or very soon after completion of translation, since the Δhns strain maintained normal levels of insAB+ transcription, messenger stability, and translation initiation but allowed amounts of InsAB+ protein production that were barely detectable by pulse-labeling.

Suppression of the InsAB+ deficit by mutations in ssrA and lon also highlight this stage in transposase production as the point at which a lack of H-NS is sensed. Nevertheless, it is not clear how these two functions might be related in InsAB+ degradation. The SsrA peptide tag elicits degradation by the ClpAP and ClpXP proteases (17), whereas we found that clpX and clpP mutations did not reduce the level of InsAB+ protein below that in wild-type cells. The spectrum of proteases to which SsrA-tagged proteins are sensitive has been extended to FtsH (18), but not to Lon. Hence, even if the SsrA peptide were often fused to InsAB+ near the latter’s 3′ terminus, it is unlikely that it would act as a direct target of Lon. An example of the more subtle interactions of proteolytic pathways is the degradation of the UmuD/UmuD’ heterodimer reported by Gonzalez et al. (16). Close to the Lon degradation signal in the N terminus of UmuD is a short peptide patch needed for ClpXP attack of UmuD’, while degradation of UmuD’ by ClpXP is necessary to expose the UmuD protein to Lon. It is possible that an interaction of nascent InsAB+ with another protein might account for the involvement of the SsrA tagging system in its stability.

Our finding that Δhns cells fail to make or maintain the

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**TABLE 4. Effect of lon mutation on transposition frequency in an hns mutant**

<table>
<thead>
<tr>
<th>lon</th>
<th>hns</th>
<th>IPTG (1 mM)</th>
<th>Transposition frequency (Spc+/Gmr-) (10^-3)</th>
<th>Cointegrate fraction (Amp+/Spc-)</th>
<th>Δhns/hns+</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>+</td>
<td>-</td>
<td>0.73</td>
<td>0.64</td>
<td>0.0062</td>
</tr>
<tr>
<td>Δ+</td>
<td>-</td>
<td>0.0045</td>
<td>0.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>2.1</td>
<td>0.81</td>
<td></td>
<td>0.022</td>
</tr>
<tr>
<td>Δ+</td>
<td>+</td>
<td>0.047</td>
<td>0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>::Tn10</td>
<td>+</td>
<td>-</td>
<td>8.9</td>
<td>0.77</td>
<td>0.026</td>
</tr>
<tr>
<td>Δ+</td>
<td>-</td>
<td>0.23</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>8.7</td>
<td>0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Δ+</td>
<td>+</td>
<td>2.6</td>
<td>0.94</td>
<td>0.30</td>
<td></td>
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</tbody>
</table>

*a +, present; -, absent.*
InsAB' protein does not exclude the possibility that H-NS also participates directly in the transposition process through binding of IS1 and target DNA or by other means. It does mean, however, that there is no evidence for the claim by Shiga et al. (39, 40) that low IS1 transposition frequencies in hns cells imply direct involvement of H-NS in a transposition complex. The experiments on which these authors based their conclusion did not include a control for the presence of the InsAB' protein.

The observation that IS1 transposase is subject to proteolysis in vivo is hardly surprising, in view of its susceptibility to protease attack in vitro (M.-C. Serre, unpubished data) and the reported instability of other transposases (e.g., that of IS903, also degraded by Lon [7]). What is unusual is the apparent protective effect of H-NS. How might H-NS help ensure the survival of InsAB' or the completion of its synthesis? Among the multitude of genes subject to repression by H-NS (19), one or more might encode functions inimical to InsAB' accumulation. Alternatively, more general physiological changes associated with H-NS deficiency, such as those stemming from diminished transcription from stringently regulated promoters (20), might activate such functions. It is also possible that H-NS acts at a posttranscriptional level. Hns mutations have been reported to affect the translation of mRNA both positively (rpoS [49]) and negatively (malT [21]) and to enhance the stability of the sigma factor RpoS (49). H-NS protein interacts directly with an RNA chaperone, StPa, protecting it from degradation by Lon protease (22), as well as with a flagellar motor protein, FlIG (8). Distinct domains of H-NS specify repression, DNA binding, and dimerization functions (46); the observation of Shiga et al. (39) that hns mutations that abolish DNA binding and repression do not reduce IS1 transposition while a mutation that prevents dimerization does argue in favor of H-NS action at the posttranscriptional level.

Simple working hypotheses include an ability of H-NS to recognize nascent InsAB' on the ribosome and chaperone it through the folding process, protecting it from Lon protease, and an ability of H-NS to counteract chaperones which modify InsAB' to promote its degradation. Further experiments are needed to assess the validity of these ideas.

Although the lon::Tn10 mutation returned transposition frequency in the Δhns strain to that of wild-type E. coli, it did not allow the frequency to rise to that seen in the hns lon strain (Table 4). It is possible that in the strain used as a donor in the mating-out assay of transposition frequency, the absence of H-NS exposes the InsAB' protein to proteases other than Lon. Alternatively, secondary effects of the combination of hns and lon mutations may interfere in some way with transposition and mask the full extent of restoration of InsAB' activity. Involvement of H-NS in the transposition process itself also might explain why transposition falls short of wild-type levels in the Δhns lon strain. The latter possibility will be best examined by analysis of the IS1 transposition mechanism in vitro using purified components.

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