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Protective Role for H-NS Protein in IS*1* Transposition

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The transposase (InsAB′) of the insertion element IS*1* 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 IS*1* 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 [^{35}S]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 *ftsH* or *clpP*, restored InsAB′ synthesis in the *hns* strain, and a mutation in *ssrA* 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 other proteins of *Escherichia coli* are needed to transform the strand transfer intermediate into the transposition products (27). More generally, host factors appear to act as modulators of transposition (reviewed in reference 4). HU strongly stimulates formation of the Mu synaptic complex in vitro (3) and presumably in vivo. IHF maintains a bend in the IS*10* ends which governs the choice of transposition pathway (41): it also stimulates transposase binding to Tn*1000* ends (47). A strong IHF binding site is present in each of the inverted termini of IS*1*, though its function is unclear (15). Fis and DnaA modulate IS*50* transposition (34). Acyl carrier protein and ribosomal protein L29 stimulate recognition of the specific Tn*7* target sequence (38). *Hns* mutations influence the Mu transposition rate in a growth medium-dependent manner (13). Since these proteins act in general to mold and compact DNA and to influence gene expression, it is not hard to imagine how they might influence the precise geometry of interacting partners or serve as the agents through which transposition responds to cell physiology.

The small (768-bp) enterobacterial transposable element IS*1* is an interesting example, because its transposition has several different outcomes: simple insertion of the element at new sites (2), formation of cointegrate molecules in which the donor replicon is fused to the target by flanking copies of the element (14, 28), deletion of DNA adjacent to the element (26, 33), inversion (6), and circle formation by precise excision (35, 45). Whether these products arise by branching of a common pathway or from distinct mechanisms is unknown.

The likelihood that accessory host proteins contribute to this diversity of transposition end products seemed high, since IS*1* is essentially simple, being composed of two partly overlapping open reading frames, *insA* and *insB*, bounded by short, imperfect terminal repeats, IRL (left end) and IRR (right end). The transposase, InsAB′, is made as a result of a low-frequency −1 translational frameshift at the sequence A6C in the overlap region (11, 36). To identify host functions that regulate IS*1* transposition, we used the SOS response induced by InsAB′ (25) as a screen for the inhibitory effects of mutations in candidate genes. Mutant alleles of most genes, including *himD* (IHF subunit) and *fis*, did not affect the SOS response to InsAB′, but a Tn*10* 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 precedents cited above, we expected that H-NS would affect IS*1* transposition by directly modulating the transposition pathway. Our results show, however, that it intervenes at another point in IS*1* 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(*srl-recA*)*306*::Tn*10* carrying the conjugative transposon target plasmid, pOX38::dTn*10* *cat* (Cm^r), or when

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TABLE 1. Plasmids used and constructed

Plasmid	Relevant characteristics[a]	Source or reference
pAPT1	ori_{p15A} *kan, lacp*::$c1857_{\lambda}$	29
pMET13	ori_{pMB1} *bla* pL_{λ}::$insA^{+}B^{+}$ Ωon-*spc*	11
pMET12	As pMET13 but *insAB′* (A7C)	11
pMET37	ori_{pMB1} *bla lacp*::*insAB′* (GA2GA3C) $lacI^{q}$ Ωon-*spc*	10
pMET35	As pMET37 but Δ*insAB′*	10
pTemp1.1	ori_{pMB1} *bla* pL_{λ}::*lacZ*	D. Zerbib
pDAG98	As pMET37 but *insAB′* Ω(codon 127::*lacZYA*)	This work
pDAG99	As pMET37 but *insAB′* Ω(codon 206::*lacZYA*)	This work
pDAG92	ori_{pMB1} *bla araC araBADp*::*insA′*::*lacZ*	This work
pMET8	ori_{pMB1} *bla* Ωon-*spc*	11
pMP3	ori_{pMB1} *bla* Ωon-*spc* $insA^{+}B^{+}$	30
pCST200	ori_{pMB1} *bla araC araBADp*::$insA^{+}B^{+}$	37
pCST400	As pCST200 but *insAB′* (GA2GA3C)	37

[a] Ωon-*spc* 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 donor carried the *lon*::Tn*10* allele, C600 Δ*recA938*::*cat* (Cm^{r}) with pOX38::*gen* (Gm^{r}). The mutations *hns*::Tn*10*, Δ*hns*::*kan*, *lon*::Tn*10*, and *ssrA*::*cat* (kindly provided by C. Gutierrez, J.-Y. Bouet, O. Fayet, and E. Roche, respectively) were introduced into these strains by bacteriophage P1-mediated transduction.

Plasmids are listed in Table 1. pMET37, which expresses *insAB′* under *lacp* control, was constructed by successive additions to a pBR322 origin fragment of restriction fragments carrying the Ωon-*spc* unit (see below), the $lacI^{q}$ gene, the *insAB′* (GA2GA3C) sequence, and the *lacp* promoter (p1 and p2); details of construction are given elsewhere (10) and are available upon request. pMET35 was made from the immediate ancestor of pMET37 by deletion of most of the IS*1* wild-type sequence to leave the last 57 nucleotides of IS*1*, including IRR.

pTemp1.1 was constructed by Didier Zerbib. A PCR fragment containing bacteriophage lambda sequence from −229 to +3 relative to the pL transcriptional start site and flanked by synthetic HindIII sites, was cleaved with HindIII and inserted at a HindIII site upstream from a promoterless *lacZ* gene, which had replaced *tetA* in a pBR322-based vector, pAP201.

pDAG98 and -99 were made by insertion of the SmaI-StuI fragment of pRS591 (42), containing *lacZYA*, into pMET37 at the MluI site (made blunt ended by incubation with DNA polymerase I Klenow fragment and deoxynucleoside triphosphates) or the PshAI site in *insB* to fuse the first 127 and 206 codons, respectively, of *insAB′* to codon 5 of *lacZ*.

pDAG92 was made by excising a fragment from pCST420 (37) containing *araC*, *araBADp*, and the first 20 bp of *insA*, using NsiI (and then blunt ending with T4 DNA polymerase) and PvuII and inserting it at the BamHI site (after blunt ending with Klenow fragment) in the proximal end of the *lacZ* gene of a pUC12-based vector, pFDX2561 (kindly provided by Caroline Welz).

pMET8 is pBR322 with Ωon-*spc* inserted at the PvuII site.

Media and growth conditions. The medium for routine growth was Luria Bertani (LB) broth supplemented with 1.5% 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 where otherwise noted. The medium for L-[^{35}S]methionine labeling of proteins was M9-mam (M9 salts with 1 μg of thiamine/ml, 0.2% glucose, and 0.5% Difco methionine assay medium). The medium for selection of Lon^{+} transductants was LB agar containing methyl methane sulfonate at 0.05%.

SOS induction. Assay of the SOS response to induction of InsAB′ expression was essentially as described previously (25). In the case of the *lacp*-controlled *insAB′*, the conditions were the same, except that cultures were grown at 37°C and induced by addition of IPTG (isopropyl-β-D-thiogalactopyranoside) to a final concentration of 0.3 mM.

Promoter::*lacZ* activity. Strains harboring plasmids that carried inducible promoters fused to *lacZ* were grown and induced under the same conditions as those used in the SOS induction assay (P_L and *lac* promoters) or the InsAB′ production assay (*araBAD* promoter) and were sampled at intervals for determination of β-galactosidase specific activity (25).

Transposition assay. The mating-out assay described by Chandler and Galas (5) was used, except for minor modifications noted in "Bacterial strains and plasmids" above. Care was taken to start donor cultures of *hns* mutants from small colonies and to monitor growth rates prior to mating so that faster-growing cultures could be eliminated. Transposition frequencies were calculated as the ratios of Sp^{r} Cm^{r} recipients to Cm^{r} recipients (simple insertions plus cointegrates) and of Ap^{r} Sp^{r} Cm^{r} to Cm^{r} (cointegrates). The efficiency of pOX38 conjugation was ~10-fold lower from an *hns* donor than from an hns^{+} donor.

InsAB′ production. Fresh pCST400 (or pCST200) transformants of MC1061 strains were grown overnight at 37°C in LB containing glucose (0.2%) and ampicillin, and the overnight cultures were diluted in fresh medium at an optical density at 600 nm (OD_{600}) of 0.05 and incubated with aeration at 30°C. The cultures were maintained in logarithmic growth for about six generations by repeated dilution to monitor the growth rate and then induced by the addition of arabinose to 1% and incubated for one more hour. Samples of known OD_{600} were chilled by mixing with cold 10 mM sodium azide, centrifuged, and resuspended in sodium dodecyl sulfate (SDS)-mercaptoethanol buffer (24). Samples equivalent to 0.05 OD_{600} units were subjected to electrophoresis and Coomassie blue staining.

Pulse-chase analysis of InsAB′ stability. Overnight cultures in M9-mam (plus ampicillin) of MC1061 hns^{+} and Δ*hns* strains freshly transformed with pCST400 were diluted in fresh medium at an OD_{600} of ~0.02, and the cultures were incubated at 37°C with aeration. At an OD_{600} of ~0.25 (late log phase), a sample was withdrawn into another culture flask (uninduced control), and arabinose was added to the rest at 1% (final concentration). After incubation for a further one-half generation time (30 min for hns^{+}; 42 min for Δ*hns*), 4.0 ml of culture was added to 20 μCi of L-[^{35}S]methionine (Amersham) (800 Ci/mmol) and incubated for 1 min before addition of unlabeled methionine to 1 mg/ml. Incubation was continued, and 0.5-ml samples were taken in ice-cold TESAz buffer (50 mM Tris, pH 8.0, 5 mM EDTA, 50 mM NaCl, 1 mM NaN_3) at the time of unlabeled methionine addition and at intervals thereafter for 1 h. The uninduced culture, as well as an induced culture of the hns^{+} strain carrying pCST200, was quenched immediately after the 1-min labeling period. The chilled samples were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) and exposure of the dried gel to a phosphorimager screen.

The proportion of the total ^{35}S protein label represented by the InsAB′ band was determined using the Tina-PCBas program (Fuji). Quantitation of a small region containing the band was carried out for all samples; the uninduced sample provided the background from which the values for the InsAB′ band were calculated. These values were then normalized by dividing each of them by the value for the total protein in its lane.

Northern hybridization analysis. The hns^{+} and Δ*hns* strains of MC1061 carrying plasmids for induction of *insAB′* expression were grown in LB at 37°C to an OD_{600} of ~0.3 and then induced by addition of IPTG or arabinose. After further incubation for 30 min, the cultures were added to phenol-$CHCl_3$ and RNA was extracted by the hot-phenol method, separated on formaldehyde agarose gels, transferred to a nylon membrane (Hybond N+; Amersham), and hybridized with a radioactively labeled DNA probe, as described previously (12). The probe was a PCR fragment composed of nucleotides 2 to 674 of the 699-bp *insAB′* fused reading frames, labeled by random priming in the presence of [α-^{33}P]dATP (3,000 Ci/mmol).

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 P_L 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 (GA2GA3C) *insAB′* and the $lacI^{q}$ repressor gene are carried on a single plasmid (pMET37). An artificial IS*1* element (omegon [Ωon]) (31) was present in both InsAB′ producer plasmids to provide ends for cleavage by transposase.

TABLE 2. Effects of *hns* mutations on induction of the SOS response by IS*1* transposase

Promoter and gene	*hns*	Presence of:		β-galactosidase sp act (Miller units)
		InsAB′	IRL + IRR	
P_L::*insAB′* (A7C) at 39°C	+	−	−	149
	+	+	−	256
	+	+	+	859
	::Tn*10*	−	−	150
	::Tn*10*	+	−	200
	::Tn*10*	+	+	264
lacp::*insAB′* (GA2GA3C) + IPTG	+	−	+	177
	+	+	+	1,900
	Δ::*kan*	−	+	149
	Δ::*kan*	+	+	240

These plasmids were introduced into an SOS reporter strain that carries a λ*imm*434 prophage with its P_L 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 P_L-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 IS*1* ends on the plasmid, since in their absence β-galactosidase rose only modestly, to 256 U (presumably through action on chromosomal IS*1* ends). Derivatives of the reporter strain carrying *hns*::Tn*10* and Δ*hns*::*kan* alleles were constructed. The SOS response to InsAB′ induction in the *hns*::Tn*10* 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 Δ*hns*::*kan* derivative. Here, full induction of *lac* promoter activity with IPTG and an *insA-insB* joint sequence on which reverse frameshifting does not occur (GA2GA3C) (11) led to higher levels of InsAB′ and an increased SOS response in *hns*$^+$ cells. Residual synthesis in the Δ*hns* mutant, however, was as low as in the *hns*::Tn*10* mutant.

The P_L and *lacp* promoters were just as active in the Δ*hns* 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 IS*1* transposition. We next tested this suggestion directly.

IS*1* transposition in *hns* mutants. Transposition frequency was measured by the mating-out assay of Chandler and Galas (5). A Δ*recA* derivative of strain C600 carrying the F′, pOX38-Cm, was transformed with the transposon donor plasmid pMET37, which carries *bla* (Ap^r), *lacp*::*insAB′* (GA2GA3C), and the repressor gene, *lacI*^{q}, as well an artificial IS*1* element (Ωon-*spc*) composed of two IRLs flanking the *aadA* (spectinomycin resistance) gene. Transposition to the F′ was measured as the fraction of F$^+$ (Cm^r) exconjugant recipients that were also Ap^r Sp^r (cointegrates) or Sp^r (cointegrates plus simple insertions). The first two lines of Table 3 show the main results. Transposition in the Δ*hns* 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 Δ*hns* strain, however, IPTG induction increased transposition ~35-fold. The *hns* mutation did not significantly affect the frequency of cointegrates relative to direct insertion events.

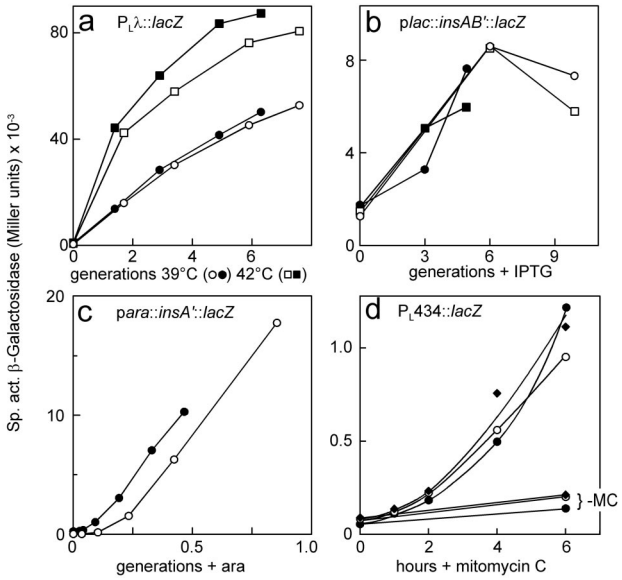

FIG. 1. Activities of promoters in *hns* mutant strains. Cultures of isogenic *hns*$^+$ (open symbols) and *hns* (solid symbols) strains growing exponentially in LB medium were shifted to inducing conditions (0 generations) and sampled at intervals for assay of β-galactosidase. (a) BR293 strains carrying pAPT1 (*cI857*) and pTemp1.1 (P_L::*lacZ*). (b) MC1061 strains carrying *lacI*^{q} 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 (○, *hns*$^+$; ●, Δ*hns*; ◆, *hns*::Tn*10*) carrying P_L434::*lacZ* (SOS reporter fusion) at *att*λ 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 Δ*hns* 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.

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^r donor plasmids which carried a natural IS*1* and Ωon-*spc* (pMP3) or Ωon-*spc* alone (pMET8); transposition from the latter plasmid depends on InsAB′ produced from chromosomal IS*1* copies and can be taken as the background for the assay. Transposition from pMP3 in the Δ*hns* 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 Δ*hns* strains reflects an authentic involvement of H-NS in IS*1* transposition. The frequencies of Ap^s Sp^r 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 Spc^r 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 Δ*hns* strain suggested that in

TABLE 3. Effects of Δ*hns* allele on IS*1* transposition frequency

Transposon donor plasmid	*hns*	IPTG (1 mM)[a]	Transposition frequency (± SD) (Spc^r/Cam^r)	*n*	Cointegrate fraction (Amp^r/Spc^r)	Δ*hns*/*hns*$^+$
pMET37 (*lacp*::*insAB′*)	+	−	1.4×10^{-3} (±0.5)	3	0.62	0.00033
	Δ	−	4.6×10^{-7} (±3.0)	3	0.60	
	+	+	1.2×10^{-3} (±0.2)	3	0.71	0.013
	Δ	+	1.6×10^{-5} (±0.7)	3	0.50	
pMP3 (wild-type IS*1*)	+		1.1×10^{-4} (±0.3)	2	0.12	0.0032
	Δ		3.5×10^{-7} (±1.4)	4	0.29	
pMET8 (no *insAB′*)	+		2.7×10^{-7} (±0.1)	2	0.93	0.56
	Δ		1.5×10^{-7} (±0.1)	4	0.95	

[a] +, present; −, absent.

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

InsAB′ production in Δ*hns* cells. Cultures of strain DLT288 (Δ*hns*) carrying pCST400, in which the *insAB′* (GA2GA3C) sequence is controlled by the *arap* 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 5) contain an abundant polypeptide of ~27 kDa that is not seen in extracts of uninduced cells (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 Δ*hns* 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 Δ*hns* 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.

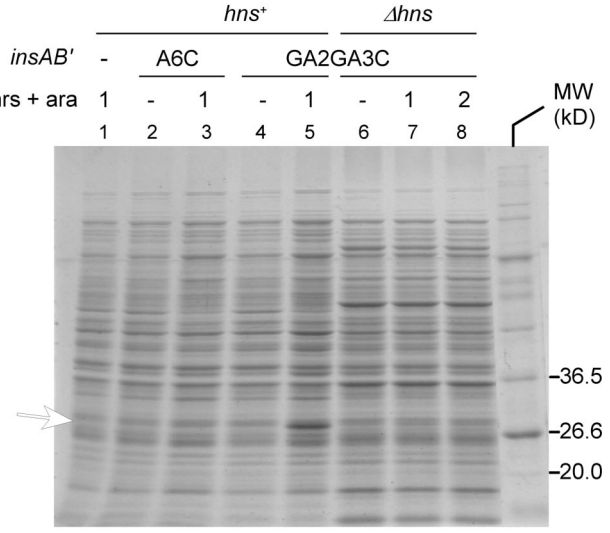

FIG. 2. IS*1* transposase (InsAB′) protein in *hns*$^+$ and Δ*hns* cells. Cultures of MC1061 strains carrying no IS*1* sequence (−), wild-type *insAB′* (A6C), or fused-frame *insAB′* (GA2GA3C) under *araC-araBADp* control, on plasmids pDAD18, pCST200, and CST400, respectively, were grown in LB medium at 30°C to late log phase. Portions of the cultures were then added to arabinose (final concentration, 1%) or left untreated (−), and incubation was continued for 1 or 2 h, as shown, before sampling for analysis of the total protein content by SDS-PAGE (12% acrylamide) and Coomassie blue staining. The arrow indicates the position of the InsAB′ band.

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 L-[^{35}S]methionine, and labeling was terminated 1 min later by the addition of excess nonradioactive methionine. Samples were withdrawn at intervals for assay of ^{35}S-labeled InsAB′ protein by SDS-PAGE and radioautography. We were obliged to observe InsAB′ against a background of labeled host polypeptides, because quantitative 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 ^{35}S-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 Δ*hns* 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 Δ*hns* 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 Δ*hns* cells.

Without a measurement of InsAB′ stability in Δ*hns* 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 Δ*hns* 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 IS*1* 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 Δ*hns* cells were comparable to, or even higher than, those in the equivalent *hns*$^+$ cells. Prentki et al. (32) reported the presence

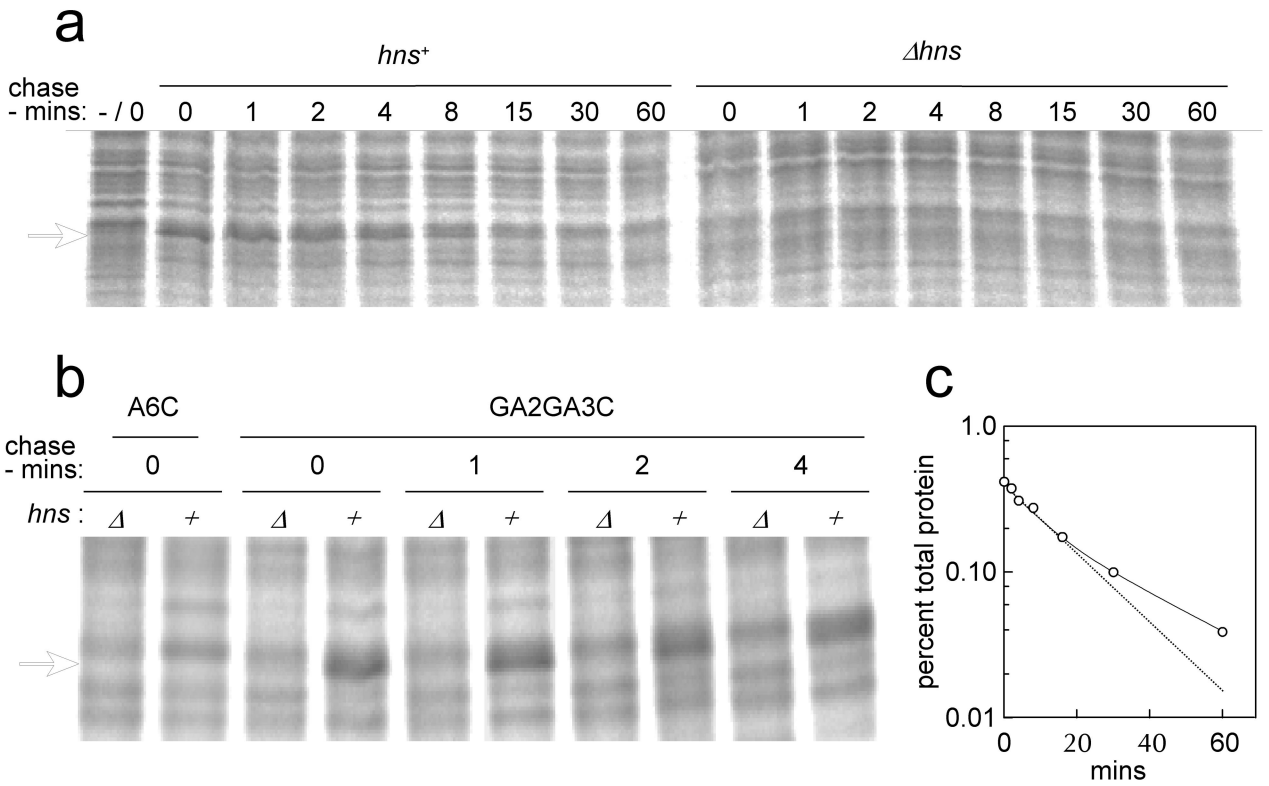

FIG. 3. Pulse-chase analysis of InsAB′ synthesis and stability. (a) Arabinose-induced cultures of *hns*$^+$ and Δ*hns* strains carrying pCST400 were labeled with L-[^{35}S]methionine for 1 min before the addition of excess unlabeled methionine. Samples removed at intervals (chase-mins) were subjected to SDS-PAGE and radioautography. −/0, uninduced *hns*$^+$ culture, labeled as described above. The high- and low-molecular-weight regions of the gel are not shown. (b) As in panel a, with the *hns*$^+$ and Δ*hns* samples from the 0- to 4-min time points paired. (c) ^{35}S-InsAB′ as a percentage of total labeled protein plotted against time after addition of unlabeled methionine. The half-life was calculated (see Materials and Methods) from the initial degradation rate, shown by the dotted line.

of a rho-dependent terminator in IS*1*; 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′ 286 nucleotides of *insB* revealed comparable amounts of IS*1*-specific RNA in the *hns*$^+$ and Δ*hns* 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 Δ*hns* 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 least 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 most of the *insAB′* mRNA is not significantly affected by the Δ*hns* 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 Δ*hns* cells. We examined the abilities of mutant alleles of known proteolysis-related genes to restore InsAB′ synthesis in the Δ*hns* strain. Figure 5a shows a Coomassie blue-stained SDS-polyacrylamide gel on which the proteins of *lon* and *ssrA* mutant derivatives of strains DLT288 (Δ*hns*) 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 Δ*hns* or *hns*::Tn*10* 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 Δ*hns* strains (20). It was therefore important to test whether the observed restoration of InsAB′ synthesis in the *lon*::Tn*10* 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 Δ*hns* *lon*::Tn*10* 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 Δ*hns* strain (Fig. 5b).

To determine whether restoration of InsAB′ synthesis by the *lon* mutation is accompanied by the return of InsAB′ function,

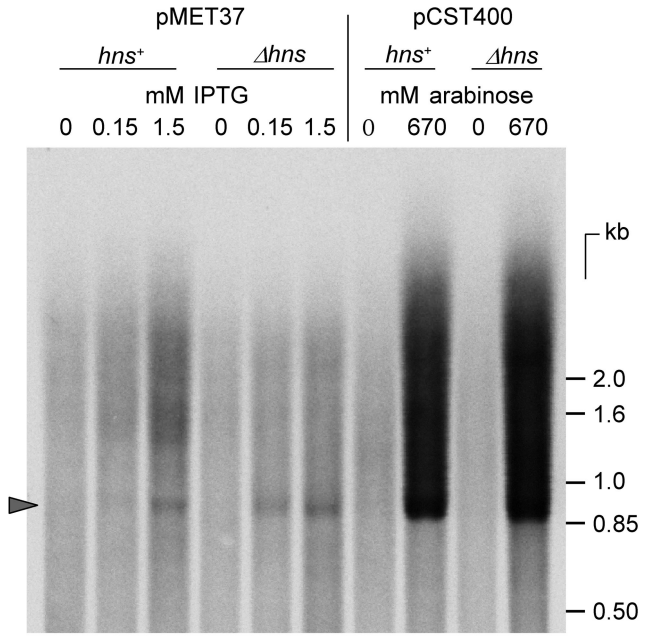

FIG. 4. Northern analysis of *insAB′* mRNA. Total RNAs extracted from cells growing exponentially in LB and induced as shown were resolved by formaldehyde-agarose gel electrophoresis, transferred to nylon membranes, and hybridized with ^{33}P-labeled *insAB′* DNA. The amount of Δ*hns* RNA loaded was 0.66 times that of *hns*$^+$ RNA (10 μg) to compensate for the lower proportion of rRNA present in *hns* mutant cells (20). The arrowhead indicates the position of the *insAB′* mRNA. The scale on the right is derived from a ^{33}P-labeled 1-kb DNA ladder (Gibco-BRL) denatured and processed with the RNAs.

transposition frequency in the Δ*hns lon*::Tn*10* strain was measured, using the mating-out system described above. The transposition frequency in the Δ*hns* 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

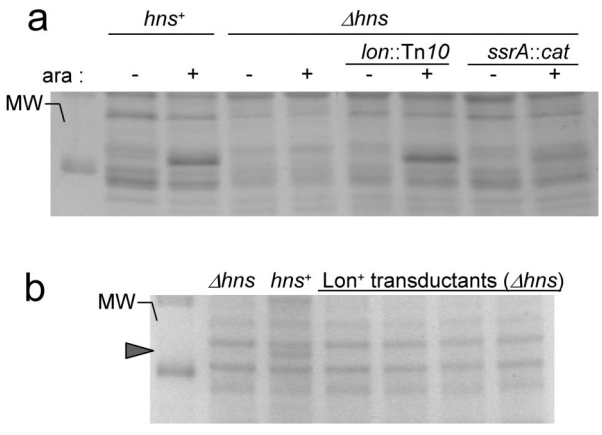

FIG. 5. InsAB′ protein in mutant derivatives of the Δ*hns* strain. (a) Cultures of the MC1061-based strains shown, carrying pCST400, were induced (+) with arabinose (ara) or not induced (−) and were analyzed as for Fig. 2. (b) As in panel a, arabinose-induced cells of four Lon$^+$ Tet^s transductants of the Δ*hns lon*::Tn*10* strain. The arrowhead indicates the position of the InsAB′ band.

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

lon	*hns*	IPTG (1 mM)[a]	Transposition frequency (Spc^r/Gm^r) (10^{-3})	Cointegrate fraction (Amp^r/Spc^r)	Δ*hns*/*hns*$^+$
+	+	−	0.73	0.64	0.0062
	Δ	−	0.0045	0.75	
	+	+	2.1	0.81	0.022
	Δ	+	0.047	0.71	
::Tn*10*	+	−	8.9	0.77	0.026
	Δ	−	0.23	0.64	
	+	+	8.7	0.73	
	Δ	+	2.6	0.94	0.30

[a] +, present; −, absent.

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 Δ*hns lon*::Tn*10* 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*::Tn*10* mutant.

DISCUSSION

We have shown that in *hns* mutants, the inability of IS*1* 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 Δ*hns* 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 Δ*hns* cells fail to make or maintain the

InsAB′ protein does not exclude the possibility that H-NS also participates directly in the transposition process through bending of IS*1* 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 IS*1* 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 IS*1* transposase is subject to proteolysis in vivo is hardly surprising, in view of its susceptibility to protease attack in vitro (M.-C. Serre, unpublished data) and the reported instability of other transposases (e.g., that of IS*903*, 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 IS*1* transposition while a mutation that prevents dimerization does argues 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*::Tn*10* mutation returned transposition frequency in the Δ*hns* 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 Δ*hns lon* strain. The latter possibility will be best examined by analysis of the IS*1* transposition mechanism in vitro using purified components.

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