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# Differential protease-mediated turnover of H-NS and StpA revealed by a mutation altering protein stability and stationary-phase survival of *Escherichia coli*

(nucleoid-associated protein/Lon-protease target/degradation signal/protein–protein interaction)

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**ABSTRACT** **The *Escherichia coli* proteins H-NS is recognized as an important component among the major nucleoid-associated proteins. In studies of *E. coli* strains with defects in H-NS, we discovered a mutant that phenotypically restored stationary-phase viability (Rsv) of such strains. The Rsv phenotype was the result of a mutation that led to severalfold higher levels of the functionally and structurally related StpA protein. This mutation was a base pair change in the *stpA* structural gene, and the amino acid substitution in the StpA protein altered its turnover properties, suggesting a role for this residue in a cleavage site for proteolysis. We determined the stability of the StpA and the H-NS proteins and found that the StpA protein was degraded relatively rapidly in strains lacking functional H-NS, whereas H-NS remained stable irrespective of the presence/absence of StpA. Using protease-deficient mutants, we obtained evidence that the Lon protease was responsible for the degradation of StpA. The differential turnover of the nucleoid-associated proteins is suggested to contribute to the regulation of their stoichiometry and ratio in terms of homo- and heteromer formation. We conclude that StpA, in contrast to H-NS, is present mainly in heteromeric form in *E. coli*.**

Enterobacteria have several nucleoid-associated proteins that are important for chromosomal organization, and the H-NS protein is among the most abundant of such proteins in *Escherichia coli*. It affects expression of genes under certain conditions, and mutations in the genes encoding H-NS and its paralogue, StpA, affect the expression of several distinct operons both positively and negatively (reviewed in refs. 1 and 2). Expression of the *hns* gene is stimulated by the cold shock protein CspA (3) and the regulatory protein FIS (4, 5), but expression has also been shown to be autoregulated (4, 6, 7). Recently, expression of H-NS was shown to be under control of the DsrA RNA, although the exact mechanism remains to be elucidated (8, 9). The H-NS protein has also been suggested to affect gene expression at the posttranscriptional level—e.g., by affecting the translation of the gene encoding $\sigma^S$ (10) and by affecting expression of the MalT protein (11).

The StpA protein has RNA chaperone activity (12), and overexpression of StpA can functionally suppress certain *hns* mutant features (13). Expression of *stpA* is negatively regulated by H-NS but stimulated by the leucine-responsive protein Lrp. Expression of *stpA* is also temperature dependent, with a lowered expression at 26°C as compared with 37°C (13). Overexpression of the StpA protein causes lowered expression of the *hns* gene (13). The H-NS and StpA proteins are able to form heterodimers *in vitro* (14), and evidence for StpA and H-NS coordinate activity at certain genes has also been found (13, 15).

In this report, we show evidence that the StpA protein is rapidly degraded in the absence of H-NS and that the degradation requires functional Lon protease. We isolated a mutant showing restored viability in stationary phase and found that it contained a mutation in the *stpA* gene encoding a more stable form of StpA.

## MATERIALS AND METHODS

**Bacterial Strains and Plasmids.** Strains are listed in Table 1. Plasmids used were pBSN157, a transcriptional *stpA-lacZ* fusion plasmid (13), and its vector pFZY1 (16).

**Growth Media and Culture Conditions.** Bacteria were grown in Luria–Bertani (LB) media (17) at 37°C with vigorous shaking (220 rpm). Growth was monitored by measuring Klett units on a Klett Summerson colorimeter, where 50 Klett units (no. KS-66, red filter) corresponds to approximately $OD_{600}$ = 0.4. Where considered necessary, antibiotics (Sigma) were used at the following concentrations: carbenicillin, 25 $\mu g \cdot ml^{-1}$; chloramphenicol, 10 $\mu g \cdot ml^{-1}$; kanamycin, 25 or 50 $\mu g \cdot ml^{-1}$; spectinomycin, 100 $\mu g \cdot ml^{-1}$; and tetracycline, 7.5 $\mu g \cdot ml^{-1}$. Plates containing 40 $\mu g \cdot ml^{-1}$ of the chromogenic $\beta$-glucoside 5-bromo-4-chloro-3-indolyl $\beta$-D-glucopyranoside (Sigma) were used to monitor the Bgl phenotype expressed by *hns* mutant derivatives.

**Stationary-Phase Survival Experiments.** Samples were taken on a daily basis from stationary-phase cultures, serially diluted in a physiological salt solution, and plated on LB plates. Viable counts were determined as colony-forming units (CFU)$\cdot ml^{-1}$ and plotted as log CFU$\cdot ml^{-1}$ versus time. The experiment was repeated two times, with similar results.

**$\beta$-Galactosidase Assay.** Samples were taken from LB cultures at 50 Klett units. $\beta$-Galactosidase reactions were assayed as in ref. 18, with the exception that we used chloroform and 0.002% sodium dodecyl sulfate (SDS) to disrupt the bacteria. Data represent the mean values from assays performed in duplicate in three separate experiments, and standard errors were calculated. The numbers of plasmid-containing cells were measured as before (13).

**Genetic Techniques.** Molecular genetic manipulations were performed essentially as described (19). Generalized bacteriophage P1 transduction was done as previously described (20). JGJ150 (*hns*::$Cm^r$) was derived from MC4100, which was transduced with P1 grown on BSN1 [*hns*::$Cm^r$ (21)]. JGJ151 (*rpoS*::Tn*10*, *hns*::$Cm^r$) was derived from RH90 (*rpoS*::Tn*10*), which was transduced with P1 grown on BSN1. JGJ152 (*hns*::$Cm^r$, *stpA60*::$Km^r$) was derived from JGJ150 that was

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Abbreviation: wt, wild type.

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Table 1. Bacterial strains

| Strain | Description | Source/ref. |
|---|---|---|
| MC4100 | *rpoS*$^+$, Δ(*argF–lac*) | 30 |
| BSNI | *hns*::Cm$^r$, | 21 |
| BSN5 | *stpA60*::Km$^r$ | 13 |
| BEU603 | *trp*::Tn*10*, Δ*hns* | 13 |
| RH90 | MC4100, *rpoS*::Tn*10* | 31 |
| JGJ150 | MC4100, *hns*::Cm$^r$ | This work |
| JGJ151 | RH90, *hns*::Cm$^r$ | This work |
| JGJ152 | JGJ150, *stpA60*::Km$^r$ | This work |
| JGJ201 | RH90, *stpA60*::Km$^r$ | This work |
| JGJ202 | JGJ151, *stpA60*::Km$^r$ | This work |
| JGJ210 | Rsv derivative of JGJ151 | This work |
| CAG18562 | *zfi-3143*::Tn*10* Km$^r$ | 22 |
| JGJ211 | JGJ210, *zfi-3143*::Tn*10*Km$^r$ | This work |
| JGJ212 | MC4100, *zfi-3143*::Tn*10* Km$^r$ StpA$_{wt}$ | This work |
| JGJ213 | MC4100, *zfi-3143*::Tn*10* Km$^r$ StpA$_{F21C}$ | This work |
| JGJ214 | JGJ150, *zfi-3143*::Tn*10* Km$^r$ StpA$_{wt}$ | This work |
| JGJ215 | JGJ150, *zfi-3143*::Tn*10* Km$^r$ StpA$_{F21C}$ | This work |
| SG20250 | MC4100, *lon*$^+$ | 23 |
| SG1041 | MC4100, *lon*-100 | 23 |
| JGJ220 | SG20250, *trp*::Tn*10* | This work |
| JGJ221 | SG20250, *trp*::Tn*10*, Δ*hns* | This work |
| JGJ222 | SG1041, *trp*::Tn*10* | This work |
| JGJ223 | SG1041, *trp*::Tn*10*, Δ*hns* | This work |
| BSN26 | MC4100, *trp*::Tn*10* | 11 |
| BSN28 | MC4100, *trp*::Tn*10*, *stpA60*::Km$^r$ | 11 |

Cm$^r$, chloramphenicol-resistant; Km$^r$, kanamycin-resistant.

transduced with P1 grown on BSN5 (*stpA60*::Km$^r$). JGJ201 (*rpoS*::Tn*10*, *stpA60*::Km$^r$) was derived from RH90 that was transduced with P1 grown on BSN5. JGJ202 (*rpoS*::Tn*10*, *hns*::Cm$^r$, *stpA60*::Km$^r$) was derived from JGJ151 that was transduced with P1 grown on BSN5. JGJ210 (Rsv derivative of JGJ151) was isolated as described in *Results*. JGJ211 (*rpoS*::Tn*10*, *hns*::Cm$^r$, *zfi-3143*::Tn*10*Km$^r$, StpA$_{F21C}$) originated from JGJ210 that was transduced with P1 grown on CAG18562 [*zfi-3143*::Tn*10*Km$^r$ (22)]. JGJ212 (*zfi-3143*::Tn*10*Km$^r$, StpA$_{wt}$) and JGJ213 (*zfi-3143*::Tn*10*Km$^r$, StpA$_{F21C}$) were derived from MC4100, JGJ214 (*zfi-3143*::Tn*10*Km$^r$, *hns*::Cm$^r$, StpA$_{wt}$) and JGJ215 (*zfi-3143*::Tn*10*Km$^r$, *hns*::Cm$^r$, StpA$_{F21C}$) originated from strain JGJ150. Strains MC4100 and JGJ150 were transduced with P1 grown on JGJ211, and transductants were isolated by selection for kanamycin resistance (i.e., the *zfi-3143*::Tn*10*Km$^r$ marker; *zfi–stpA* cotransduction frequency was about 25%). To investigate whether the transductants either received the *stpA* allele containing the base pair substitution or whether they still possessed the wild-type (wt) allele, we used primer 40 (5′-GGGGGTACCGAAATAATCTCGCGCAGGACTG-3′) and primer 41 (5′-GGGGTCGACCTTTGTTGGTGCCGGGTTACTG-3′) to PCR amplify the *stpA* gene. Thereafter, we subjected the obtained fragment to *Eco*RI digestion. This digestion confirmed that the *stpA* gene of strain JGJ212 and JGJ214 contained the *Eco*RI site, whereas the *stpA* alleles in JGJ213 and JGJ215 did not. JGJ220 (*trp*::Tn*10*) and JGJ221 (*trp*::Tn*10*, Δ*hns*) were derived from strain SG20250 [*lon*$^+$ (23)], which had been transduced with P1 grown on BEU603 [*trp*::Tn*10*, Δ*hns* (13)]. Transductants were selected for growth on tetracycline plates and subsequently tested with respect to Bgl phenotype. The *hns*$^+$ strain showed a negative Bgl phenotype and the *hns* strain showed a positive Bgl phenotype. Similarly, strains JGJ222 (*trp*::Tn*10*) and JGJ223 (*trp*::Tn*10*, Δ*hns*) originated from strain SG1041 [*lon* (23)], which had been transduced with P1 grown on BEU603.

***In Vivo* Protein Stability Experiment.** To determine the intracellular stability of StpA and H-NS we used a technique described in ref. 24. Protein stability was monitored after the protein synthesis had been inhibited by the addition of spectinomycin (100 $\mu$g·ml$^{-1}$) to bacterial cultures grown to 50 Klett units in LB medium at 37°C. Samples to be analyzed by Western blotting were removed at indicated times.

**Gel Electrophoresis and Western Blotting.** Bacteria were pelleted and resuspended in sodium dodecyl sulfate (SDS)/polyacrylamide gel electrophoresis (PAGE) sample buffer. The samples (sample loading was normalized according to determination of cell mass) were subjected to SDS/PAGE (15% polyacrylamide concentration) and thereafter blotted onto a 0.2-$\mu$m-pore-size poly(vinylidene difluoride) transfer membrane (Bio-Rad) by use of a semidry blotting apparatus. H-NS and StpA were purified essentially as described previously (6, 12). Polyclonal rabbit antisera directed against purified StpA or H-NS were used as the primary antibodies in Western blot analysis. To further increase the specificity of the StpA antiserum, it was adsorbed against a lysate of a *stpA* mutant strain. Further visualization followed the method described in ref. 11, where the membrane was developed with AttoPhos substrate (JBL Scientific, Northridge, CA) and scanned with the STORM system (Molecular Dynamics). Measurement of the StpA protein and its half-life were performed with the IMAGEQUANT program (Molecular Dynamics).

## RESULTS

**Isolation of Mutants Restoring Stationary-Phase Viability to *hns* Strains.** Our earlier studies with an *E. coli* strain carrying mutations in the *hns* and *stpA* loci indicated that there might be effects on growth and viability (13). To further examine the role of H-NS and StpA on bacterial growth and viability we tested a number of different strains with such mutations in prolonged growth experiments using LB liquid medium at 37°C. Relevant antibiotics were included to ensure that the original mutations were retained. Viability was monitored by viable counts on solid medium without antibiotics (Fig. 1*A*). While *hns*$^+$ strains showed a decrease in viability of about two orders of magnitude during extended incubation at stationary phase, the different *hns* mutants showed a strikingly different behavior: The strain containing mutations in *hns* and *rpoS* (JGJ151) and the strain carrying mutations in *hns*, *stpA,* and *rpoS* (JGJ202) displayed reduced viability immediately after they had reached the stationary phase. Within 2–3 days the viability was reduced by 6–8 orders of magnitude in such cultures (Fig. 1*A*). A similar reduction in viability was exhibited by *rpoS*$^+$, *hns* mutant strains (JGJ150, JGJ152) after about 7–8 days. Cultures of strains lacking both H-NS and StpA appeared to completely lose viability upon prolonged incubation. Interestingly, after the initial large reduction in colony-forming units, cultures of the strain JGJ151 showed resumed viability after another 2–3 days, when a subpopulation appeared and reached a cell density as high as the wt strain (Fig. 1*A*). At day 8, we isolated single-cell colonies from JGJ151 cultures, and a selected isolate used in further studies was denoted JGJ210. Isolates showing restored viability will hereby be referred to as restored stationary-phase viability (Rsv). The Rsv phenotype was not observed with strains also carrying a mutation in the *stpA* gene (e.g., JGJ152, JGJ202).

To study the growth of the Rsv isolate, and to investigate whether it, unlike its parental strain JGJ151, could maintain high viability for several days in stationary phase, we reinoculated strain JGJ210 in LB medium at 37°C and monitored its viability in a comparison with the parental strain and a wt strain (MC4100). As shown in Fig. 1*B*, JGJ210 did not show the same reduction in viability as the parental strain JGJ151 after the first few days, but remained stable at approximately the same level as the wt strain.

**The Amount of StpA Protein Is Elevated in Rsv Isolates.** One possible explanation for our results would be that the expression of StpA was increased and therefore able to com-

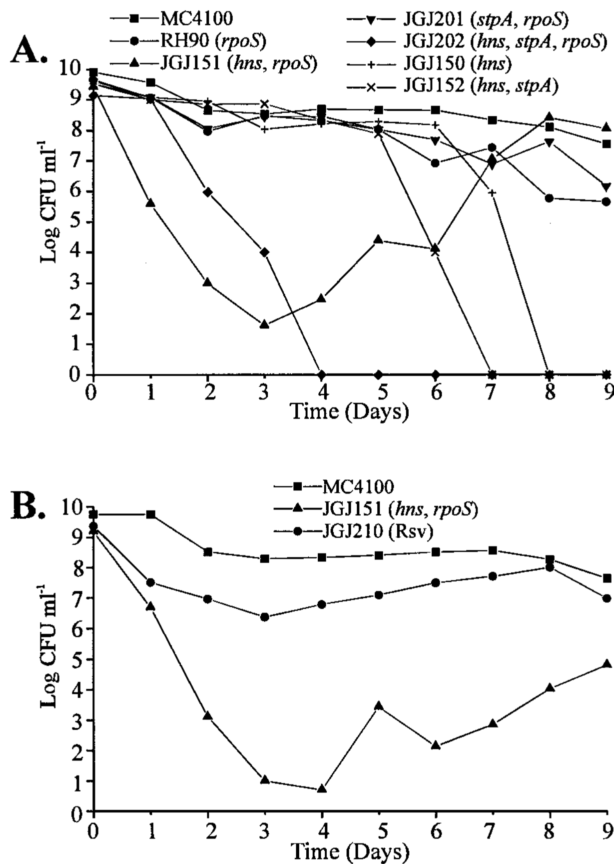


FIG. 1. (*A*) Viability test of strains in stationary phase at 37°C. (*B*) Viability test of the Rsv isolate JGJ210. All strains were grown in LB medium at 37°C to stationary phase (day zero). Thereafter, samples were taken each day for viable count determinations and results were plotted on a logarithmic scale versus time. CFU, colony-forming units.

plement the deficiency in H-NS. To investigate this possibility we performed Western blotting analysis with antisera directed against StpA and showing very low cross-reactivity to H-NS. As seen in Fig. 2, the level of StpA increased approximately 3-fold in JGJ210 as compared with the parental strain JGJ151.

The finding of an elevated level of StpA protein prompted us to study if this was caused by an increase at the transcriptional level. We therefore used a low-copy-number *stpA-lacZ* transcriptional fusion plasmid to measure if an increased expression could be detected from the *stpA* gene in trans (13), and the results are summarized in Table 2. The level of *stpA-lacZ* expression was lowered in the Rsv strain JGJ210 as compared with its parental strain JGJ151, and nearly as low as in the strain containing functional H-NS. This suggests that the

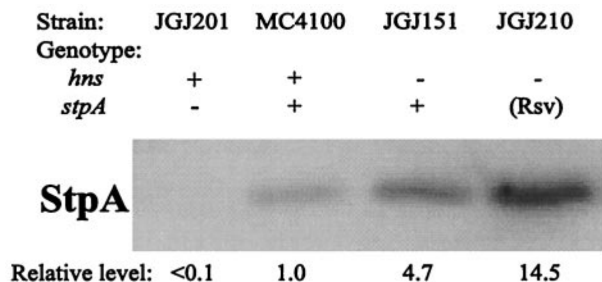


FIG. 2. Quantitative determination of StpA protein content. Western blotting was performed with total cell extract from the following strains: MC4100 (wt), JGJ151 (*hns*, *rpoS*), JGJ210 (Rsv), and JGJ201 (*rpoS*, *stpA*) included as negative control.

Table 2. Expression of an *stpA-lacZ* operon fusion

| Strain | β-Galactosidase | |
|---|---|---|
| | pFZY1 (vector) | pBSN157 (*stpA-lacZ*) |
| MC4100 (wt) | 3 ± 0.3 | 307 ± 16 |
| JGJ151 (*hns*, *rpoS*) | 3 ± 0 | 1,111 ± 130 |
| JGJ210 (Rsv) | 3 ± 0 | 442 ± 71 |

β-Galactosidase activity was monitored as described in the text. Data are given as mean ± SEM of three separate experiments.

increased level of the StpA protein in strain JGJ210 was not due to increased transcription of the *stpA* operon. Instead, the results indicate that the higher levels of StpA in JGJ210 led to repression of its own transcription.

**The Restored Viability Phenotype Results from a Single Amino Acid Substitution (F21C) in the StpA Protein.** To further analyze the mutation conferring increased levels of StpA, we sequenced the *stpA* locus. We could not find any differences in the promoter region of JGJ210 as compared with the parental strain JGJ151 (data not shown). However, in the structural gene of *stpA*, we detected one base pair substitution, changing codon 21 to TGC from TTC (Fig. 3*A*). At the amino acid level, this substitution converted the phenylalanine at position 21 to a cysteine in strain JGJ210 (Fig. 3*B*). With genetic methods, we transferred the mutation to other strains. Because the substitution disrupted an *Eco*RI site we could easily confirm the presence of the specific mutation by *Eco*RI digestion of PCR-amplified chromosomal DNA (see *Materials and Methods*). We concluded that the Rsv phenotype of JGJ210 was due to the mutation in the *stpA* gene resulting in $StpA_{F21C}$.

**The StpA Protein Is Unstable in the Absence of H-NS.** The finding that StpA levels were altered by an amino acid substitution led us to consider that it affected protein turnover. We therefore studied StpA protein stability in different genetic backgrounds. We used a method (24) in which protein synthesis is inhibited by the addition of 100 $\mu$g·ml$^{-1}$ spectinomycin

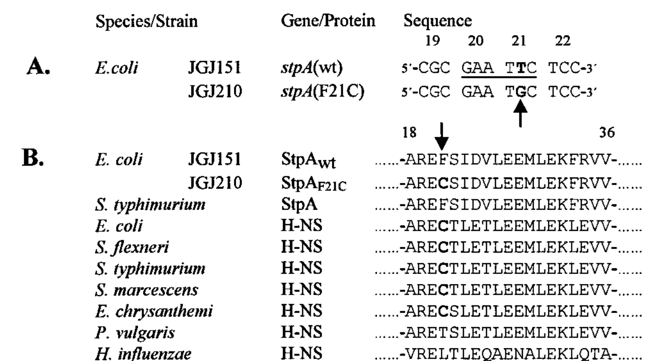


FIG. 3. Sequence analysis of the *stpA* gene from the Rsv mutant. (*A*) DNA sequence alignment of four codons, showing the single base pair difference in boldface between the *stpA* sequence of strain JGJ151 and the Rsv isolate JGJ210. An arrow points out the base pair substitution, and the *Eco*RI site in the wt sequence is underlined. The codon positions are shown above the sequences. (*B*) Multiple peptide sequence alignment of StpA and H-NS peptides from several species. Boldface letters highlight the cysteine residue at position 21 (also indicated by arrow). The numbers above the sequences indicate relative position, where 1 is the N-terminal methionine. Accession numbers for the protein sequences are as follows: StpA *E. coli*, Sw P30017; StpA *Salmonella typhimurium*, GB AF009363; H-NS *E. coli*, Sw P08936; H-NS *Shigella flexneri*, Sw P09120; H-NS *Salmonella typhimurium*, Sw P17428; H-NS *Serratia marcescens*, Sw P18955; H-NS *Erwinia chrysanthemi* EMBL X89444; H-NS *Proteus vulgaris*, Sw 18818; and H-NS *Haemophilus influenzae*, Sw P43831. EMBL, GB, and Sw refer to the European Molecular Biology Laboratory, GenBank, and Swiss-Prot databases, respectively.

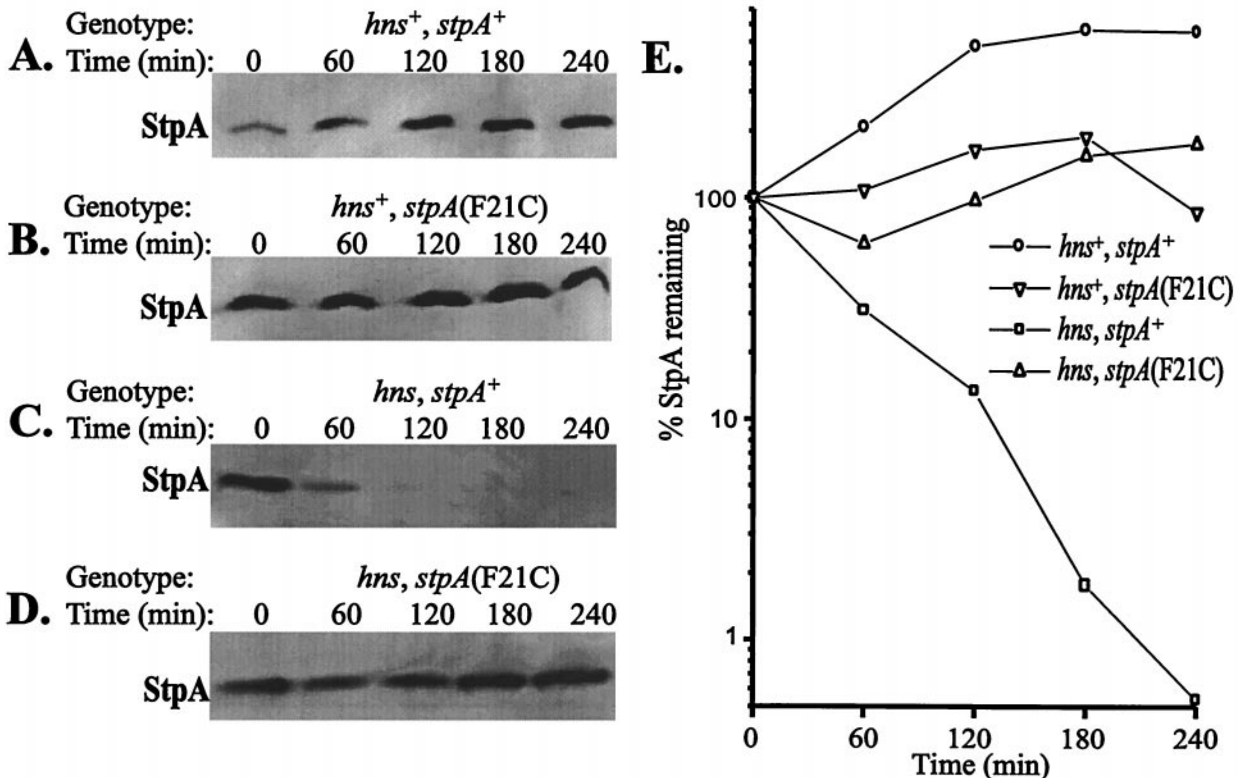


FIG. 4. H-NS-dependent stability and turnover of StpA. The relative stability of StpA was measured after protein synthesis was inhibited as described in the text. StpA stability was measured in the following strains: (*A*) JGJ212 (*hns*$^+$, *stpA*$^+$); (*B*) JGJ213 [*hns*$^+$, *stpA*(F21C)]; (*C*) JGJ214 (*hns*, *stpA*$^+$); and (*D*) JGJ215 [*hns*, *stpA*(F21C)]. (*E*) Quantitative determinations of StpA from the different experiments in *A–D* plotted as a function of time.

and samples are removed at different times (see *Materials and Methods*). Immunodetection was performed with antisera recognizing StpA but not H-NS (cf. Fig. 2). As seen in Fig. 4 *A* and *B*, the StpA protein was stable throughout the duration of the experiment in a genetic background ensuring functional H-NS. Even 4 hr after the addition of spectinomycin, approximately the same level of StpA was detected. However, the level of $StpA_{wt}$ protein in strains lacking functional H-NS was reduced to a larger extent already 60 min after the addition of spectinomycin (Fig. 4*C*). Quantitative measurements of the level of StpA at the different time points were used to estimate the half-life of the protein, and $StpA_{wt}$ showed a half-life of about 35 min under these conditions (Fig. 4*E*). Interestingly, no degradation could be detected in a strain containing $StpA_{F21C}$ but lacking H-NS (Fig. 4*D*). Even after 4 hr, the level of $StpA_{F21C}$ was approximately the same as at the onset of spectinomycin addition. The finding that StpA was unstable in the absence of H-NS prompted us to determine whether there was a decline in StpA levels during stationary phase of the strains that lost viability. As shown in Fig. 5, the StpA levels declined in strain JGJ151 (*hns*, *rpoS*) soon after entry into stationary phase. The decline in StpA levels was independent of *rpoS,* since we obtained similar results (data not shown) with strain JGJ150 (*hns*). In the case of the Rsv isolate JGJ210, the StpA levels remained constant in the stationary phase (data not shown).

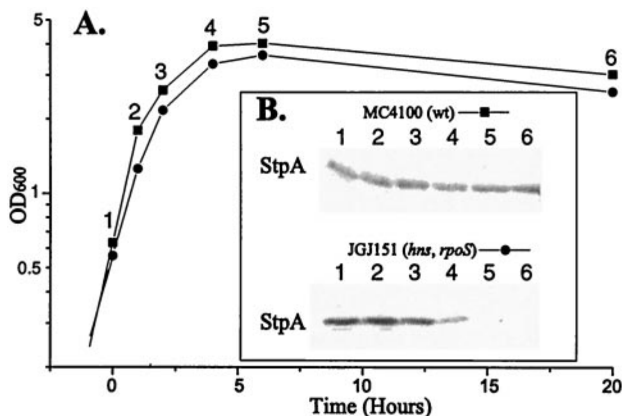


FIG. 5. Determination of StpA levels during entry into stationary phase. (*A*) Growth of strains MC4100 (wt) and JGJ151 (*hns*, *rpoS*) into stationary phase (the time point of 20 hr corresponds to day 0 in Fig. 1). Samples were removed at indicated times (1–6). (*B*) Western blotting to detect StpA was performed with total cell extracts from samples taken at times indicated in *A*.

We performed similar studies of H-NS protein stability. The level of H-NS protein was unaffected both in a wt strain and in a *stpA* mutant strain during the 4 hr after the addition of spectinomycin (Fig. 6 and data not shown). Apart from the StpA proteins in *E. coli* and *S. typhimurium*, none of the other so-far-identified H-NS-like proteins in different bacteria has a phenylalanine at position 21 (Fig. 3*B*). However, as in the case of mutant $StpA_{F21C}$, a cysteine at position 21 is also present in the H-NS proteins of five different bacterial species, among them the H-NS protein of *E. coli* (Fig. 3*B*).

**Turnover of StpA Is Lon Protease Dependent.** To test if some specific protease was responsible for the degradation of StpA, we examined the level of StpA protein in protease-deficient strains. In *hns*$^+$, *stpA*$^+$ strains lacking the OmpT or Lon proteases, we were not able to detect any significant difference in the steady-state levels of StpA protein according to Western blot analysis (data not shown). To examine the possible involvement of the Lon protease in the turnover of StpA, we introduced an *hns* allele into otherwise isogenic *lon*$^+$ and *lon* mutant strains and tested StpA protein stability by using the same approach described above. As seen in Fig. 7, the

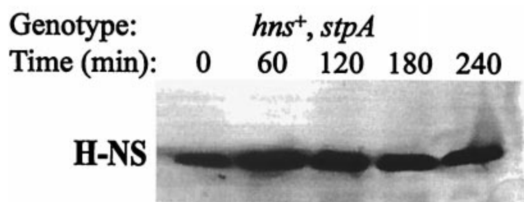


FIG. 6. Stability of H-NS protein in the absence of StpA. The relative amount of H-NS in strain BSN28 (*hns*$^+$, *stpA*) was measured after protein synthesis was inhibited as described in the text.

StpA protein had completely disappeared 160 min after the addition of spectinomycin in the case of *hns*, *lon*$^+$ cells (Fig. 7, lane 4). In contrast, strains also lacking Lon protease had a nearly unaffected level of StpA throughout the duration of the test (Fig. 7, lane 8). As detected before, the level of StpA was not affected in *hns*$^+$ strains (Fig. 7, lanes 2 and 6). These results provided strong evidence that the StpA protein is a substrate for the Lon protease and that StpA in complex with H-NS is protected from proteolysis. We subsequently tested if a *lon* mutation *per se* could alter the survival of an *hns* mutant strain during stationary phase. The results (data not shown) indicated that the *lon* mutation could not restore viability to the *hns* mutant strain. This finding also suggested that we might not expect to find Lon-defective mutants among Rsv isolates.

## DISCUSSION

The present data show that there is differential turnover of the nucleoid-associated proteins H-NS and StpA. The StpA protein was subjected to relatively rapid degradation *in vivo* when H-NS protein was absent (Fig. 4*C*). On the other hand, no such effect was observed in the case of the H-NS protein, which appeared quite stable in the absence of StpA (Fig. 6). In the presence of H-NS, however, turnover of StpA was not detected during the 4-hr-long experiment (Fig. 4*A*). These results suggest that there are very low levels of StpA monomers and homodimers normally *in vivo* because they would be degraded rapidly. Apparently, the bacteria have different mechanisms to ensure appropriate levels of H-NS and StpA to maintain certain cellular properties. At the transcriptional level there is both autoregulatory control of their expression and cross-talk regulation between the *hns* and *stpA* genes (4, 6, 7, 13). While neither the regulationally active nor the protease-sensitive molecular form of StpA (i.e., monomers and oligomers, respectively) is determined, it is evident that the levels of StpA will be governed by both synthesis and degradation. Fig. 8 shows a schematic summary of the molecular forms and turnover of StpA and H-NS and their regulation. On the basis of the present evidence, we conclude that StpA is present mainly in heteromeric complexes with H-NS in *E. coli*. We postulate that such heteromers are functionally different from, and presumably have other targets than, H-NS or StpA homomers.

The observation that the StpA protein remained stable in a *lon* mutant strain during a long period of time strongly supports the conclusion that Lon is the only protease degrading StpA. There is an interesting similarity in our findings to earlier studies on the nucleoid-associated HU proteins in *E. coli* (25). It was shown that one of the two HU proteins, HU1 (also referred to as HU$\beta$ or HupB) is subject to Lon-dependent degradation (25). However, there are also differences between the features shown by the HU proteins and features shown by the H-NS/StpA proteins. The two HU subunits occur in homodimer and heterodimer forms with varying composition during the growth cycle, and the heterodimer formation was reported to be required for long-term survival in stationary phase (26). In the case of H-NS/StpA our present data show that formation of heteromers *per se* is not essential for viability, but that homomers of either protein H-NS$_{wt}$ and StpA$_{F21C}$ may ensure long-term viability (Fig. 1).

Survival of *E. coli* in stationary phase is known to depend on RpoS, and there are presumably many alterations in cell physiological features when bacteria adapt to the altered conditions (27). Mutants that may take over stationary-phase cultures were earlier shown to be altered in the *rpoS* gene (28). The very drastic reduction in viability shown here with *hns*, *stpA* mutants suggests that products from some gene(s) affected by H-NS/StpA *per se* cause cell death. In absence of RpoS the cells lost viability at once, whereas in the case of *rpoS*$^+$ derivatives the loss of viability was delayed (Fig. 1). The reason for this difference is not known, but evidently the combination of H-NS and RpoS deficiency made the bacteria particularly unfit to the stationary phase. In the present study, we did not observe any Rsv revertants with the *rpoS*$^+$, *hns* strain after the prolonged incubations. The reason for this failure is unclear, but perhaps progressive changes in the culture medium (e.g., nutrient depletion and pH alterations) did not allow for efficient growth of such putative mutants. The fact that StpA$_{F21C}$ restored viability in the absence of H-NS implied that it was a gain-of-function mutation. The results indicated that the StpA$_{F21C}$ protein was able to repress the transcription of *stpA* (Table 2) and therefore was still functional in gene regulatory systems despite the amino acid substitution. Our results furthermore suggest that the in-

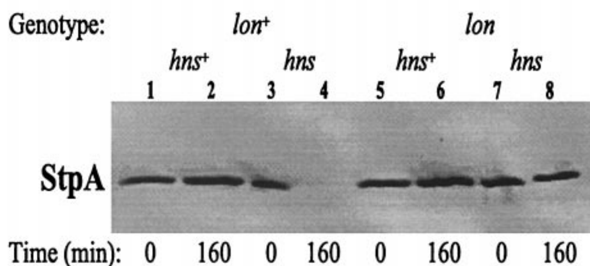


FIG. 7. StpA stability in Lon-protease-deficient *E. coli*. Measurement of StpA stability in JGJ220 (*hns*$^+$, *lon*$^+$), JGJ221 (*hns*, *lon*$^+$), JGJ222 (*hns*$^+$, *lon*), and JGJ223 (*hns*, *lon*) strains. The relative amount of StpA was monitored by Western blotting after protein synthesis was inhibited.

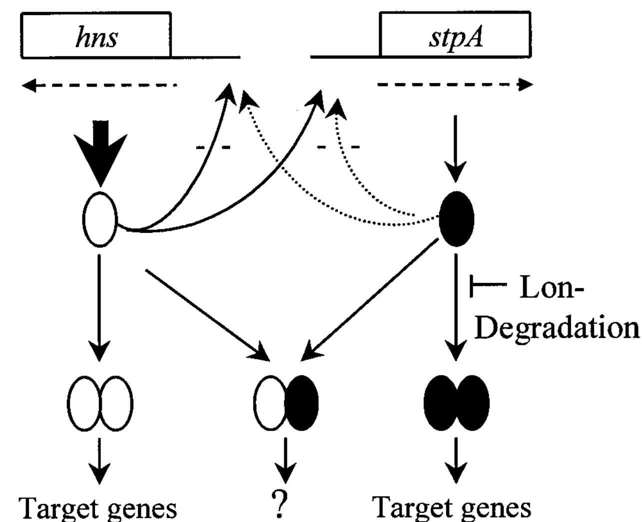


FIG. 8. Schematic summary of molecular forms and turnover of StpA and H-NS. Lon-protease-dependent StpA degradation is indicated in the case of StpA in accordance with the present data. Coordinate regulation at the transcriptional level has been demonstrated earlier (13, 15).

creased level of StpA in a $StpA_{F21C}$ strain (Fig. 2) was caused by the inability of the Lon protease to degrade $StpA_{F21C}$ (Fig. 4*D*). This could be because the mutation changes the conformation of StpA to a more compact, stable form. Another explanation would be that the mutation makes the StpA protein uncleavable by the Lon protease. This latter possibility is further supported by the comparison between the StpA sequence, and the Lon protease consensus cleavage site proposed (29): $\Phi X_{3-4}LS(L,X)X_5SX\Phi$, in which $\Phi$ represents a hydrophobic side chain. This consensus fits well with the StpA sequence: $\Phi X_4LX_8\Phi$ where the first $\Phi$ is at position 21 (Fig. 3*B*). The conversion of a phenylalanine to a cysteine in $StpA_{F21C}$ results in a polar instead of a hydrophobic amino acid at the first residue of the consensus. This alteration could reduce the interaction between StpA and Lon. The isolation of the $StpA_{F21C}$ mutant protein provides an example in which loss of the Lon protease substrate phenotype is genetically selected because of its essential physiological importance. It remains to be elucidated if there is a specific growth phase or condition when StpA forms homodimers to some greater extent. Also, the question about correlation with Lon expression is of interest. Further work concerning the interaction between $StpA_{wt}$ and $StpA_{F21C}$ with the Lon protease will, we hope, shed more light on the molecular action of the Lon protease and the degradation signal(s) in such substrate proteins. Evidently, the present system offers good selective possibilities for genetic analysis of such signals and determinants.

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1. Atlung, T. & Ingmer, H. (1997) *Mol. Microbiol.* **24,** 7–17.
2. Williams, R. M. & Rimsky, S. (1997) *FEMS Microbiol. Lett.* **156,** 175–185.
3. La Teana, A., Brandi, A., Falconi, M., Spurio, R., Pon, C. L. & Gualerzi, C. O. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10907–10911.
4. Falconi, M., Higgins, N. P., Spurio, R., Pon, C. L. & Gualerzi, C. O. (1993) *Mol. Microbiol.* **10,** 273–282.
5. Falconi, M., Brandi, A., La Teana, A., Gualerzi, C. O. & Pon, C. L. (1996) *Mol. Microbiol.* **19,** 965–975.
6. Dersch, P., Schmidt, K. & Bremer, E. (1993) *Mol. Microbiol.* **8,** 875–889.
7. Ueguchi, C., Kakeda, M. & Mizuno, T. (1993) *Mol. Gen. Genet.* **236,** 171–178.
8. Lease, R. A., Cusick, M. E. & Belfort, M. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 12456–12461.
9. Majdalani, N., Cunning, C., Sledjeski, D., Elliott, T. & Gottesman, S. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 12462–12467.
10. Yamashino, T., Ueguchi, C. & Mizuno, T. (1995) *EMBO J.* **14,** 594–602.
11. Johansson, J., Dagberg, B., Richet, E. & Uhlin, B. E. (1998) *J. Bacteriol.* **180,** 6117–6125.
12. Zhang, A., Derbyshire, V., Salvo, J. L. & Belfort, M. (1995) *RNA* **1,** 783–793.
13. Sondén, B. & Uhlin, B. E. (1996) *EMBO J.* **15,** 4970–4980.
14. Williams, R. M., Rimsky, S. & Buc, H. (1996) *J. Bacteriol.* **178,** 4335–4343.
15. Zhang, A., Rimsky, S., Reaban, M. E., Buc, H. & Belfort, M. (1996) *EMBO J.* **15,** 1340–1349.
16. Koop, A. H., Hartley, M. E. & Bourgeois, S. (1987) *Gene* **52,** 245–256.
17. Bertani, G. (1951) *J. Bacteriol.* **63,** 293–300.
18. Miller, J. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
19. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
20. Willets, N. S., Clark, A. J. & Low, B. (1969) *J. Bacteriol.* **97,** 244–249.
21. Forsman, K., Sondén, B., Göransson, M. & Uhlin, B. E. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9880–9884.
22. Singer, M., Baker, T. A., Schnitzler, G., Deischel, S. M., Goel, M., Dove, W., Jaacks, K. J., Grossman, A. D., Erickson, J. W. & Gross, C. A. (1989) *Microbiol. Rev.* **53,** 1–24.
23. Trisler, P. & Gottesman, S. (1984) *J. Bacteriol.* **160,** 184–191.
24. Geuskens, V., Mhammedi-Alaoui, A., Desmet, L. & Toussaint, A. (1992) *EMBO J.* **11,** 5121–5127.
25. Bonnefoy, E., Almeida, A. & Rouviere-Yaniv, J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7691–7695.
26. Claret, L. & Rouviere-Yaniv, J. (1997) *J. Mol. Biol.* **273,** 93–104.
27. Hengge-Aronis, R. (1996) in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M. & Umbarger, H. E. (Am. Soc. Microbiol., Washington, DC), 2nd. Ed., pp. 1497–1512.
28. Zambrano, M. M., Siegele, D. A., Almiron, M., Tormo, A. & Kolter, R. (1993) *Science* **259,** 1757–1760.
29. Gottesman, S. & Maurizi, M. R. (1992) *Microbiol. Rev.* **56,** 592–621.
30. Casadaban, M. J. (1976) *J. Mol. Biol.* **104,** 541–555.
31. Hengge-Aronis, R. & Fischer, D. (1992) *Mol. Microbiol.* **6,** 1877–1886.