

Identification of a Turnover Element in Region 2.1 of *Escherichia coli* σ^{32} by a Bacterial One-Hybrid Approach

Markus Obrist^{1,2} and Franz Narberhaus^{1,2*}

Institut für Mikrobiologie, Eidgenössische Technische Hochschule, Zürich, Switzerland,¹ and Lehrstuhl für Biologie der Mikroorganismen, Ruhr-Universität Bochum, Bochum, Germany²

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Induction of the heat shock response in *Escherichia coli* requires the alternative sigma factor σ^{32} (RpoH). The cellular concentration of σ^{32} is controlled by proteolysis involving FtsH, other proteases, and the DnaKJ chaperone system. To identify individual σ^{32} residues critical for degradation, we used a recently developed bacterial one-hybrid system and screened for stabilized versions of σ^{32} . The five single point mutations that rendered the sigma factor more stable mapped to positions L47, A50, and I54 in region 2.1. Strains expressing the stabilized σ^{32} variants exhibited elevated transcriptional activity, as determined by a *groE-lacZ* fusion. Structure calculations predicted that the three mutated residues line up on the same face of an α -helix in region 2.1, suggesting that they are positioned to interact with proteins of the degradation machinery.

The heat shock response is a universal reaction in nature to defend cells against temperature-induced damage. Heat stress causes proteins to unfold and aggregate. In the gram-negative bacterium *Escherichia coli*, a heat shock regulon comprised of more than 30 heat shock genes is induced after a temperature upshift. Most of the encoded heat shock proteins are molecular chaperones (e.g., DnaK, DnaJ, GroEL, and GroES) or proteases (e.g., FtsH, Lon, and HslVU) (reviewed in references 8 and 32).

Transcription of the heat shock regulon is initiated by the alternative sigma factor σ^{32} (RpoH). The σ^{32} level is tightly controlled, mainly at the translational and posttranslational levels. Translational regulation requires a secondary structure in the coding region of the *rpoH* mRNA, which blocks the ribosomal binding site at physiological temperatures (33, 20). At heat shock temperatures (e.g., 42°C), the secondary structure melts, allowing the ribosome to interact with the ribosomal binding site of *rpoH* mRNA to initiate translation (19).

At the posttranslational level, the activity and stability of σ^{32} are regulated. About 50 σ^{32} molecules are found per cell at 30°C (26). This number increases immediately after heat shock to approximately 1,000 molecules. Controlled proteolysis of σ^{32} involves different proteases (e.g., FtsH, Lon, and HslVU) (11, 13, 29) and a network of molecular chaperones, including DnaKJ and GroESL (6, 10).

DnaK and its cochaperone DnaJ interact with σ^{32} at physiological temperatures, depleting the freely available sigma factor pool. This interaction renders σ^{32} susceptible to FtsH-mediated proteolysis. During heat stress, DnaK and DnaJ are titrated away from σ^{32} by unfolded or aggregated proteins. Free σ^{32} interacts with core RNA polymerase and induces transcription of the heat shock genes (3, 7). Shutoff of the heat shock response starts after 4 to 6 min at a heat stress temperature. As soon as sufficient amounts of chaperones are synthe-

sized, the heat shock sigma factor is redirected to degradation, reducing the amount of σ^{32} to the normal level (25, 26).

The exact mechanism of σ^{32} degradation is unknown. Aiming at isolation of σ^{32} derivatives that are stable against proteolysis, we used a recently established one-hybrid system. In this system the adenylate cyclase (AC) of *Bordetella pertussis* is used to complement an AC-deficient (*cya*) *E. coli* strain. The catalytic AC fragment, which synthesizes cAMP, comprises two subdomains called T18 and T25, which are connected via a flexible linker. This linker can be replaced by a protein of interest. If the linker is susceptible to proteolysis, the two subdomains are separated and no cAMP is produced. A stable hybrid construct produces cAMP, which can be easily detected (e.g., by a *lacZ* reporter system) (5, 15).

To isolate stabilized σ^{32} derivatives, we performed random mutagenesis on *rpoH* by error-prone PCR. The mutagenized PCR fragments were introduced between the AC subdomains, and the constructs were transformed in *cya E. coli* cells. Cells expressing stabilized σ^{32} -AC hybrids produced red colonies (Cya⁺) on MacConkey agar plates, whereas constructs susceptible to proteolysis produced white colonies (Cya⁻). All single-point mutations in *rpoH* that resulted in stabilization of σ^{32} clustered in region 2.1 of the sigma factor.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains used in this study are listed in Table 1. All strains were grown in Luria-Bertani medium (24). Antibiotics and chemicals were added as follows: ampicillin, 200 μ g/ml; chloramphenicol, 20 μ g/ml; kanamycin, 30 μ g/ml; nalidixic acid, 15 μ g/ml; and isopropyl-thio- β -D-galactoside (IPTG), 1 mM (final concentration), unless indicated otherwise). *E. coli* DH5 α was grown at 37°C, *E. coli* DHM1 and C600 and the *ftsH1* mutant were grown at 30°C, and the *E. coli* Δ *rpoH* mutant was grown at 25°C.

Plasmids and recombinant DNA techniques. Plasmids used in this study are shown in Table 1. DNA manipulations were performed by using standard protocols (24). To construct pEC5352 encoding the AC-RpoH fusion downstream of a *lac* promoter, an 870-bp fragment containing *E. coli rpoH* was amplified from pEC5217 using primers MO3 (5'-ACTCCGCTAGCCATATGACTGACAAAA TGCAAAGTTAGC-3'; restriction site underlined) and MO4 (5'-CACCAGG TACCCTCGAGCGCTTCAATGGCAGCAGC-3'). The PCR fragment was digested with NheI and KpnI and cloned into pKAcP5, replacing the p5 fragment. Selected *rpoH* point mutations were transferred from pEC5352 derivatives into pEC5217, replacing the wild-type MluI-PstI fragment. To construct

* Corresponding author. Mailing address: Lehrstuhl für Biologie der Mikroorganismen, Ruhr-Universität Bochum, NDEF 06/783, D-44780 Bochum, Germany. Phone: 49 (234) 32 23100. Fax: 49 (234) 32 14620. E-mail: franz.narberhaus@rub.de.

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant genotype or phenotype ^a	Reference or source
<i>E. coli</i> strains		
DH5 α	<i>supE44</i> Δ <i>lacU169</i> (ψ 80 <i>lacZ</i> Δ M15) <i>hsdR17</i> <i>recA1</i> <i>gyrA96</i> <i>thi1</i> <i>relA1</i>	24
DHM1	<i>cya-854</i> <i>recA1</i> <i>gyrA96</i> (Nal) <i>thi1</i> <i>hsdR17</i> <i>spoT1</i> <i>rfbD1</i> <i>glnV44</i> (AS)	D. Ladant
C600	<i>thr-1</i> <i>leu-6</i> <i>thi-1</i> <i>supE44</i> <i>lacY1</i> <i>tonA21</i> λ^-	Laboratory collection
<i>ftsH1</i>	<i>thr-1</i> <i>leu-6</i> <i>thi-1</i> <i>supE44</i> <i>lacY1</i> <i>tonA21</i> λ^- <i>ftsH1</i> (Ts) <i>fts1372</i> (Ts)	30
Δ <i>rpoH</i>	MG1655 <i>rpoH120::kan</i> <i>zhg-21::Tn10</i>	35
Plasmids		
pKACp5	pKAC derivative carrying p5, an HIV protease cleavage site, between codons 224 and 225 of the catalytic domain of the <i>B. pertussis</i> adenylate cyclase, Km ^r	5
pCD951	pKAC carrying <i>rpoH</i> N-terminal fragment M1-M156, Km ^r	4
pCD953	pKAC carrying <i>rpoH</i> C-terminal fragment T142-A284, Km ^r	4
pEC5352	pKACp5 derivative, p5 replaced by <i>E. coli</i> <i>rpoH</i> , Km ^r	This study
pEC5378	pEC5352 derivative coding for AC-RpoH (I54F), Km ^r	This study
pEC5379	pEC5352 derivative coding for AC-RpoH (A50), Km ^r	This study
pEC5380	pEC5352 derivative coding for AC-RpoH (L47Q), Km ^r	This study
pEC5381	pEC5352 derivative coding for AC-RpoH (N83D), Km ^r	This study
pEC5382	pEC5352 derivative coding for AC-RpoH (H107L), Km ^r	This study
pEC5383	pEC5352 derivative coding for AC-RpoH (I54T), Km ^r	This study
pAR145	pHSG575 vector carrying <i>ftsH</i> , Cm ^r	30
pAR145b	pAR145 lacking <i>ftsH</i> , Cm ^r	This study
pEC5217	pUC18 carrying <i>E. coli</i> <i>rpoH</i> , Ap ^r	21
pEC5356	pEC5217 derivative coding for RpoH (A50), Ap ^r	This study
pEC5357	pEC5217 derivative coding for RpoH (L47Q), Ap ^r	This study
pEC5358	pEC5217 derivative coding for RpoH (N83D), Ap ^r	This study
pEC5359	pEC5217 derivative coding for RpoH (H107L), Ap ^r	This study
pEC5360	pEC5217 derivative coding for RpoH (I54F), Ap ^r	This study

^a HIV, human immunodeficiency virus.

pAR145b, the *ftsH* gene was deleted from pAR145 by NdeI restriction and religation.

One-hybrid screening. Error-prone PCR of pEC5352 was performed with primers MO17 (5'-GACATGTTCCGCCATTATGC-3') and MO18 (5'-GATTTTCCACAACAAGTCG-3') using *Taq* polymerase and (i) standard reaction conditions, (ii) an elevated dATP concentration (16 mM instead of 2 mM), and (iii) addition of 0.2 mM MnCl₂ (24, 34). NheI/KpnI-digested PCR fragments were cloned into pKACp5 cut with the same enzymes, replacing the p5 fragment. Transformed *E. coli* DHM1 cells were screened on MacConkey agar plates with maltose as the only carbon source as described by Karimova et al. (14) and in a user manual provided by D. Ladant. Plasmids from red colonies potentially expressing stabilized RpoH constructs were sequenced using primers MO17 and MO18.

In vivo degradation assay and immunoblot analysis. *E. coli* C600 and *E. coli* *ftsH1* cells carrying pEC5352 were grown to an optical density at 600 nm of 0.4 to 0.6 at 30°C. The cultures were shifted to 42°C, and expression of AC-RpoH-WT was induced with IPTG for 15 min. Chloramphenicol (200 μ g/ml) was added, and samples were taken and frozen in liquid nitrogen. *E. coli* Δ *rpoH* cells carrying a pEC5217 derivative were grown to an optical density at 600 nm of 0.4 to 0.6 at 25°C prior to *rpoH* induction with IPTG for 15 min. Chloramphenicol was added, and samples were taken and frozen in liquid nitrogen. Cells were harvested, washed with 0.9% NaCl, treated with 2 mg/ml lysozyme in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) for 5 min before sodium dodecyl sulfate (SDS) loading dye was added, and boiled for 10 min. Equivalent amounts of protein (10 μ g) were separated on 12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Hybond-C; Amersham). RpoH proteins were detected using an anti-RpoH antibody (polyclonal rabbit anti-RpoH antibody; 1:3,000 dilution) and a secondary antibody [goat anti-rabbit immunoglobulin G(H+L)-horseradish peroxidase conjugate; Bio-Rad; 1:3,000 dilution], followed by chemiluminescence detection (SuperSignal; Pierce). X-ray films were scanned with a Molecular Dynamics PhosphorImager, and RpoH bands were quantified using the Quantity One program (version 4.5.2; Bio-Rad).

Other methods. SDS-polyacrylamide gel electrophoresis (24) and β -galactosidase assays (18) were performed as described previously. *E. coli* DHM1 and *E. coli* Δ *rpoH* cultures for β -galactosidase assays were grown in the presence of 0.5 mM IPTG.

RESULTS

Genetic screen to study σ^{32} degradation. A recently developed one-hybrid system to study protease activity (5) was adapted to explore σ^{32} degradation. The principle is illustrated in Fig. 1. The reporter system relies on the concept of monitoring cAMP production in an adenylate cyclase-negative (*cya*) *E. coli* strain that is provided with a plasmid encoding both catalytic subdomains of the AC from *B. pertussis*. The two subdomains, called T25 and T18, are connected via a flexible linker region, which can be replaced by the protein of interest,

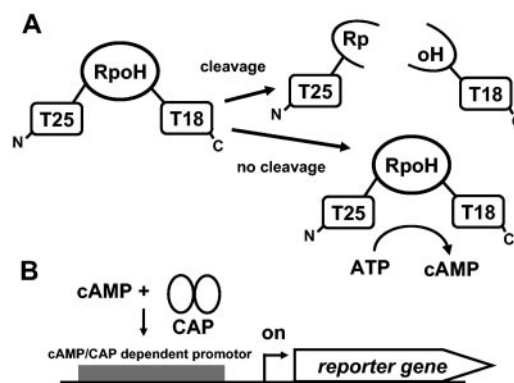


FIG. 1. Principle of the one-hybrid system. (A) cAMP is produced only when a stable linker is present between T25 and T18. (B) Detection of cAMP by a catabolite gene activator protein (CAP)-controlled reporter gene.

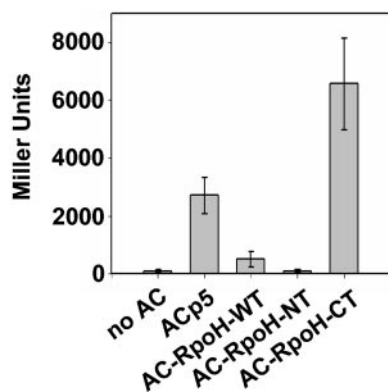


FIG. 2. β -Galactosidase activity resulting from different AC hybrids in *E. coli* DHM1. The error bars indicate standard deviations from three independent assays.

in our case σ^{32} (RpoH) (Fig. 1A). Intact hybrid constructs produce cAMP, which can be detected easily (e.g., by monitoring cAMP-dependent *lacZ* expression) (Fig. 1B). If the hybrid construct carries a protease-sensitive linker, the separated subdomains are unable to catalyze cAMP production.

To provide proof of principle, *E. coli rpoH* was cloned between T25 and T18 as described in Materials and Methods, leading to AC-RpoH-WT. ACp5, a construct carrying a proteolytically stable peptide of the human immunodeficiency virus Gag/Pol protein (5), and two recently described constructs carrying either the N-terminal region (AC-RpoH-NT) or the C-terminal region (AC-RpoH-CT) of RpoH (4) were used as controls.

The *lac* operon served as a reporter system, and β -galactosidase assays were performed (Fig. 2). The negative control (DHM1 without plasmid; *Cya*⁻ phenotype) showed some residual activity, which was stimulated 20- to 30-fold by the presence of an intact AC hybrid (ACp5; *Cya*⁺ phenotype). β -Galactosidase activity was significantly reduced when the p5 linker was replaced by RpoH, suggesting susceptibility to proteolysis. The presence of the N-terminal half of RpoH reduced β -galactosidase activity even further, whereas the construct carrying the C-terminal half of RpoH was highly active, supporting the proposal that degradation occurs via the N-terminal region (2, 1, 4).

To demonstrate proper expression of the hybrid proteins, the presence of AC-RpoH-WT, AC-RpoH-NT, and AC-RpoH-CT was examined by immunoblot analysis using anti-RpoH sera (data not shown). Two different approaches were used to examine whether the AC-RpoH-WT fusion was a substrate of the FtsH protease. First, the fusion was expressed in *E. coli* DHM1 in the absence or presence of plasmid pAR145 expressing FtsH. Production of additional FtsH in the cell reduced the β -galactosidase activity (Fig. 3A), suggesting that the fusion was cleaved. Second, expression of the fusion protein was monitored by immunoblot analysis in the temperature-sensitive *ftsH1* strain. When protein synthesis was inhibited by addition of chloramphenicol and the protease was inactivated by a shift to 42°C for 10 min, the AC-RpoH-WT levels remained constant (Fig. 3B). In contrast, the fusion was degraded in the presence of normal FtsH in *E. coli* C600.

In summary, we concluded (i) that the AC-RpoH fusion is

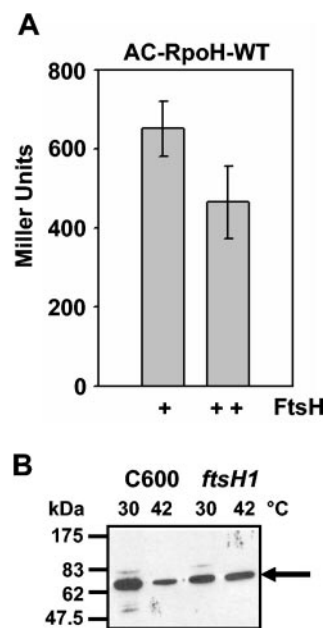


FIG. 3. Turnover of the AC-RpoH-WT fusion by FtsH. (A) AC-RpoH-WT-derived β -galactosidase activity in *E. coli* DHM1 in the presence of control plasmid pAR145b (normal FtsH levels are indicated by one plus sign) or FtsH expression plasmid pAR145 (elevated FtsH levels are indicated by two plus signs). (B) Immunodetection of the AC-RpoH-WT fusion (indicated by the arrow) in *E. coli* C600 and the *ftsH1* strain by use of anti-*E. coli* RpoH serum. Protein synthesis was blocked, and samples were taken before (30°C) and 10 min after a shift to 42°C.

degraded by FtsH, (ii) that degradation does not proceed from either end of RpoH as the ends are blocked by T25 and T18, and (iii) that the degradation signal resides in the N-terminal region of RpoH.

Screening for stabilized mutants of σ^{32} . As an additional readout system to facilitate screening on solid media, we chose the cAMP-dependent *mal* operon and provided maltose as the only carbon source in MacConkey agar plates. In line with the β -galactosidase results, colonies harboring stable AC hybrids turned red, whereas unstable fusions led to white colonies (Fig. 4).

To narrow down the region responsible for degradation in σ^{32} , we designed a random mutagenesis approach, as described in Materials and Methods (Fig. 4). Of approximately 2,000 colonies, 43 had a deep red color. Sequence analysis revealed that most *rpoH* variants carried multiple point mutations or deletions. Seven candidates, all derived from the standard PCR, carried single point mutations. Remarkably, the mutations clustered in region 2, and five of them were located in region 2.1 from residues 47 to 54 (Fig. 5). In a β -galactosidase assay, all point mutation AC-RpoH variants showed 3- to 13-fold-elevated activity compared to AC-RpoH-WT (Fig. 6), thus indicating enhanced stability.

Stabilized σ^{32} proteins are functional. To test sigma factor activity and stability of the mutated RpoH proteins devoid of the AC subdomains, selected single point mutations were transferred into the wild-type *rpoH* gene as described in Materials and Methods. Five *rpoH* alleles expressing RpoH-L47Q,

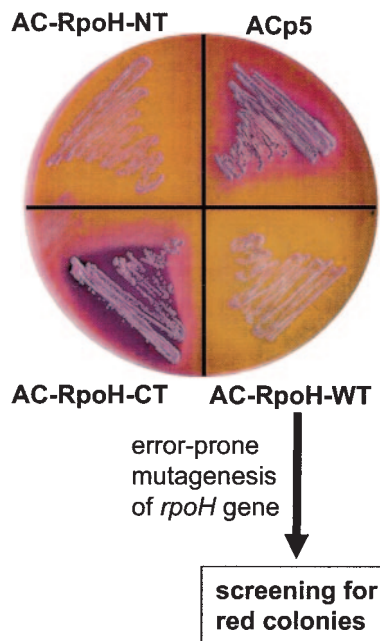


FIG. 4. Phenotypic appearance of *E. coli* DHM1 strains expressing AC hybrids. Transformants were plated on MacConkey agar plates containing maltose as the only carbon source and incubated at 30°C for 36 h. The mutagenesis and screening strategy starting from the AC-RpoH-WT protein is shown at the bottom.

-A50V, -I54F, -N83D, and -H107L were constructed. The RpoH derivatives were first tested for sigma factor function. Plasmids carrying the respective point mutated *rpoH* genes were transformed into an *E. coli* $\Delta rpoH$ strain carrying an RpoH-dependent *groE-lacZ* fusion. β -Galactosidase assays demonstrated that all RpoH variants were fully active sigma factors (Fig. 7). RpoH-L47Q, -A50V, -I54F, and -N83D showed elevated activities that were between 130 and 170% of the wild-type RpoH activity.

To assess the predicted enhanced stability of the RpoH derivatives, degradation assays with plasmid-encoded *rpoH* variants were performed in the $\Delta rpoH$ strain as outlined in

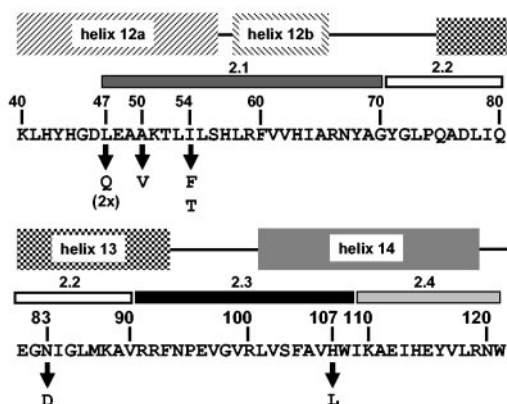


FIG. 5. All single point mutations obtained in this study cluster in region 2 of *E. coli* RpoH. AC-RpoH-L47Q was obtained independently twice. The secondary structure prediction was adapted from the study of Malhotra et al. (16).

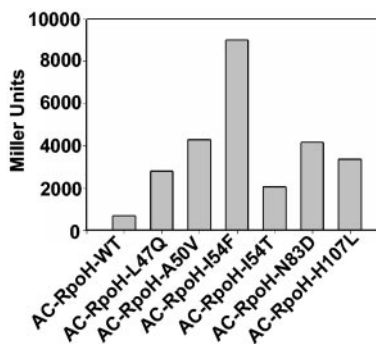


FIG. 6. cAMP-dependent *lacZ* expression mediated by AC-RpoH constructs with single point mutations in *E. coli* DHM1 cells.

Materials and Methods. RpoH-WT displayed a half-life of approximately 2 min under these conditions (Fig. 8). Unexpectedly, RpoH-N83D and RpoH-H107L were not stabilized significantly and showed wild-type-like or only slightly enhanced stability. RpoH-A50V and RpoH-L47Q had half-lives that were two- to three-fold longer than that of RpoH-WT. The half-life of RpoH-I54F was estimated to be increased at least 10-fold.

DISCUSSION

Application of the one-hybrid system. It was demonstrated that degradation of σ^{32} by the FtsH protease occurs via the N-terminal half of the sigma factor (see below). In order to pin down individual σ^{32} residues important for this process, we decided to adapt a recently established one-hybrid system (5). A hybrid between full-length RpoH and *B. pertussis* AC fragments T25 and T18 resulted in low β -galactosidase activity and white colonies on MacConkey agar plates containing maltose, presumably because the sigma factor was accessible to proteolysis. Degradation of the hybrid was shown to be mediated by the FtsH protease. Assuming that stabilized RpoH-AC hybrids would be red on MacConkey agar plates containing maltose, we mutagenized the entire *rpoH* gene. Approximately 2% of all colonies obtained were red, but most of them contained multiple mutations. Interestingly, the mutations in all seven candidates carrying only single nucleotide exchanges clustered in region 2 of the sigma factor. Five of these seven mutations

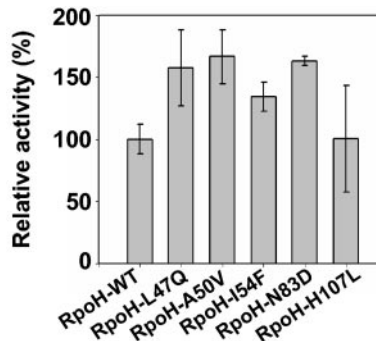


FIG. 7. Sigma factor activity of RpoH derivatives carrying single point mutations in *E. coli* $\Delta rpoH$ β -galactosidase activity which originated from a *groE-lacZ* promoter.

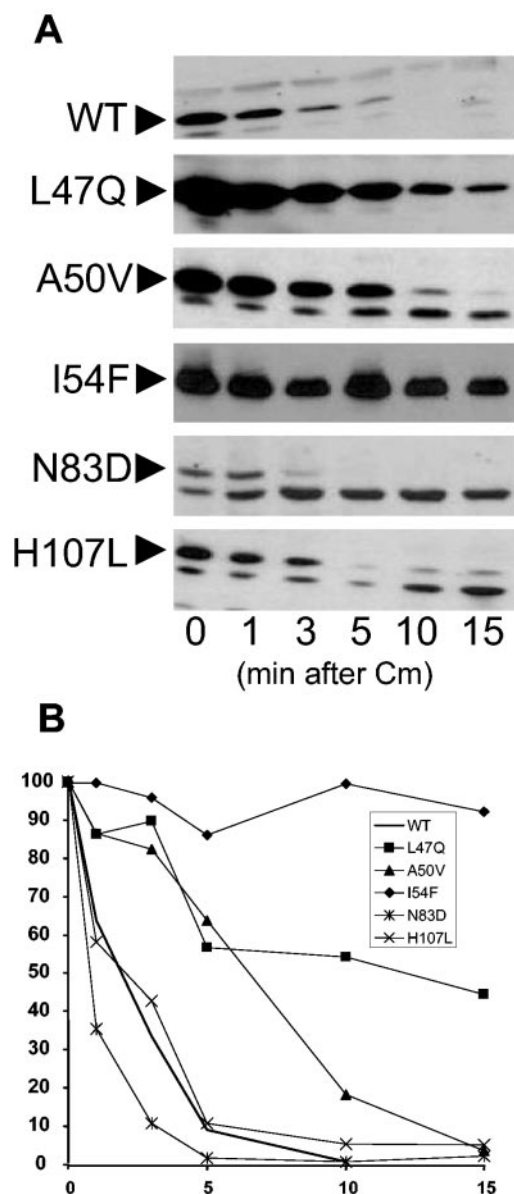


FIG. 8. Stability of RpoH derivatives carrying single point mutations. (A) Degradation of RpoH proteins as monitored by immunoblot analysis after protein synthesis was stopped by chloramphenicol (Cm) addition. (B) Quantification of RpoH degradation. Average values from three independent experiments are shown. WT, wild type.

indeed rendered RpoH stable. Why the N83D and H107L variants showed elevated β -galactosidase activity in the one-hybrid systems but were normally degraded in the absence of the AC fragments remains unclear. Despite these two false-positive candidates it is evident that the one-hybrid screen is a powerful approach for identification of protein regions susceptible to proteolysis.

The usefulness of the one-hybrid system is underlined by the fact that another entirely different screening procedure resulted in the same set of stabilized σ^{32} variants. Very recently, Horikoshi et al. (12) screened for hyperactive σ^{32} proteins, arguing that stabilized sigma factors would increase transcrip-

tion of heat shock genes. The initial screening involved approximately 10 times more clones (20,000 versus 2,000) than our approach. Candidates with apparently higher σ^{32} activity indeed were stabilized and contained the mutations L47Q, A50S, and L55Q. An additional screening using a different genetic background revealed that mutations in K51 and I54 also stabilized the sigma factor. The importance of region 2.1 for σ^{32} stability was thus confirmed by two independent approaches, one directly aiming at stabilized variants and the other screening for enhanced sigma factor activity.

Region 2.1 of RpoH. Prior to our analysis, region 2 had already been implicated in σ^{32} degradation. Swapping of fragments between *E. coli* σ^{32} and the comparatively stable *Bradyrhizobium japonicum* RpoH₁ protein showed that an element between residues 36 and 122 of *E. coli* RpoH is critical for proteolysis (2). Proteolysis did not require the N-terminal end since truncated versions were degraded normally. The one-hybrid results entirely agree with these results. AC-RpoH-WT was susceptible to proteolysis, although both termini were blocked by T25 and T18. The possibility of a role for the C terminus of σ^{32} in proteolysis has been excluded (28). Experiments with AC fusions to RpoH-NT and RpoH-CT further supported the hypothesis that the N-terminal region of σ^{32} is the target for degradation (4) (Fig. 2). Measuring Cy3- σ^{32} degradation by a fluorescence polarization assay demonstrated that σ^{32} degradation proceeds from the N terminus to the C terminus (22).

The one-hybrid approach narrowed down an important turnover element in RpoH to a few amino acids in region 2.1. Secondary structure predictions and modeling of the σ^{32} structure on the basis of the solved crystal structure of a fragment of the housekeeping sigma factor suggest that region 2.1 comprises two α -helices (16, 21). The mutations L47Q, A50V, and I54F are located in the first α -helix and line up on the same face of the helix (Fig. 9A).

Stabilization of the mutated σ^{32} factors might have various reasons. Enhanced affinity for the RNA polymerase might sequester the sigma factor away from the degradation pathway. However, this possibility was discarded by Horikoshi et al. after they transferred two stabilizing mutations into an *rpoH* allele known to confer reduced affinity to RNA polymerase (12). Combination of the mutations did not change the apparently low affinity to RNA polymerase (as determined by DnaK and GroEL levels).

Another reason might be an altered interaction with components of the degradation machinery (i.e., either the DnaKJ chaperones or FtsH itself). Both reduced and increased affinity for DnaK or DnaJ might stabilize the sigma factor. A reduced interaction with the targeting factors would prevent delivery of σ^{32} to the FtsH protease. On the other hand, a stronger interaction with the chaperones would probably protect it from further processing. A previous peptide scanning approach demonstrated that σ^{32} displays multiple contact sites for DnaK (17). Strikingly, a region from H44 to P74 was found to have high affinity for DnaK. In particular, three 13-mers (L47 to R59, A50 to V62, and L53 to A65), each including at least one of our mutated amino acids, bound DnaK tightly.

Further screening of peptide libraries revealed a characteristic DnaK recognition motif comprised of a hydrophobic core of four or five residues flanked by basic residues. The most

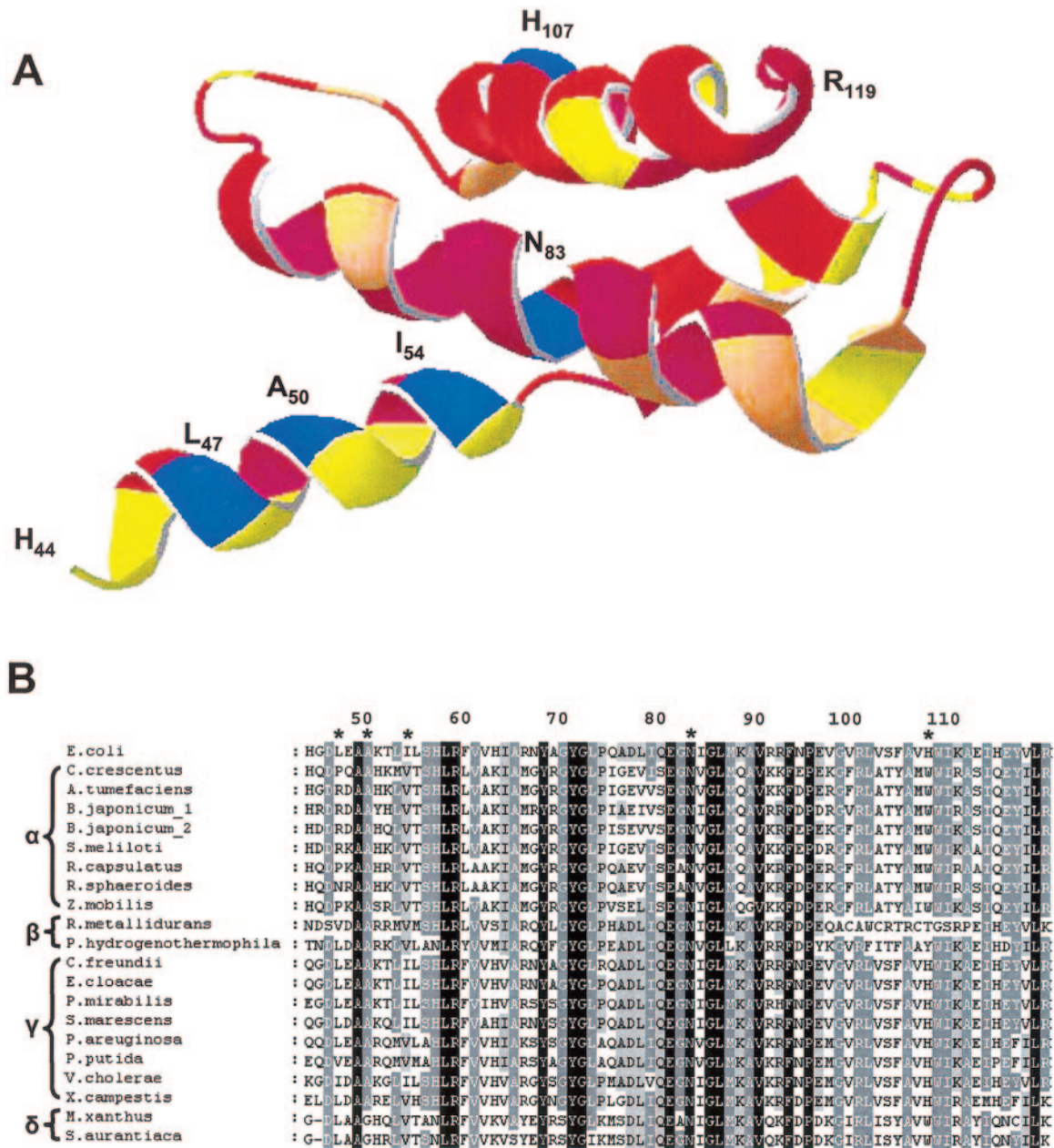


FIG. 9. Structure model and sequence alignment of region 2 from different proteobacteria. (A) Backbone ribbon diagram of a subfragment of *E. coli* σ^{32} constructed with the Swiss PDB viewer (9) and adapted from a previous study (21). The colors indicate the degree of sequence conservation according to the alignment in panel B. Variable residues are yellow, poorly conserved residues (pale gray background in panel B) are orange, highly conserved residues (dark gray background in panel B) are red, and entirely conserved residues (black background in panel B) are magenta. The five residues identified by two-hybrid screening are blue. (B) Alignment of RpoH region 2 from 19 bacterial species performed with CLUSTAL W (27). Members of different proteobacterial subdivisions are grouped as indicated on the left. The numbering refers to the numbering for the *E. coli* RpoH protein. Mutated residues are indicated by asterisks.

frequently occurring core residues are Leu, Ile, Val, Phe, and Tyr (23). The LIL sequence around residue 54 of σ^{32} (Fig. 5 and 9B) clearly resembles such a characteristic core region. It is tempting to speculate that one reason for the 10-fold-elevated stability of the *B. japonicum* RpoH₁ protein (31) is that the equivalent region is LVT (Fig. 9B). It is interesting that mutations in residues 48, 49, 51, 52, and 53 of *E. coli* σ^{32} did not affect proteolysis (12). We propose that residues 47, 50,

and 54, which line up on the same face of an α -helix in region 2.1, function as important turnover elements in the heat shock sigma factor and might be critically involved in the interaction with DnaK.

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