Dynamic interplay between antagonistic pathways controlling the σ^{32} level in *Escherichia coli*

Miyo T. Morita*, Masaaki Kanemori, Hideki Yanagi, and Takashi Yura†

HSP Research Institute, Kyoto Research Park, Shimogyo-ku, Kyoto 600-8813, Japan

Edited by Carol A. Gross, University of California, San Francisco, CA, and approved February 17, 2000 (received for review November 15, 1999)

The heat-shock response in *Escherichia coli* depends primarily on the transient increase in the cellular level of heat-shock sigma factor σ^{32} encoded by the *rpoH* gene, which results from both enhanced synthesis and transient stabilization of normally unstable σ^{32}. Heat-induced synthesis of σ^{32} was previously shown to occur at the translation level by melting the mRNA secondary structure formed within the 5′ coding sequence of *rpoH* including the translation initiation region. The subsequent decrease in the σ^{32} level during the adaptation phase has been thought to involve both shutoff of synthesis (translation) and destabilization of σ^{32}-mediated by the DnaK–DnaJ chaperones, although direct evidence for translational repression was lacking. We now show that the heat-induced synthesis of σ^{32} does not shut off at the translation level by using a reporter system involving translational coupling. Furthermore, the apparent shutoff was not observed when the synthesis rate was determined by a very short pulse labeling (15 s). Examination of σ^{32} stability at 10 min after shift from 30 to 42°C revealed more extreme instability ($t_{1/2}$=20 s) than had previously been thought. Thus, the dynamic change in σ^{32} stability during the heat-shock response largely accounts for the apparent shutoff of σ^{32} synthesis observed with a longer pulse. These results suggest a mechanism for maintaining the intricate balance between the antagonistic pathways: the *rpoH* translation as determined primarily by ambient temperature and the turnover of σ^{32} as modulated by the chaperone (and presumably protease)-mediated autogenous control.

Most organisms respond to heat or other stresses by transiently inducing molecular chaperones and other heat-shock proteins (HSPs) to cope with stress-induced protein damage (1). When *Escherichia coli* cells are exposed to modest heat shock by a shift from 30 to 42°C, the synthesis of HSP increases for several minutes (induction phase) and gradually decreases (adaptation phase) to reach a new steady-state level within 20–30 min. The induction results from a rapid but transient increase in the cellular level of σ^{32} (the *rpoH* gene product) which directs RNA polymerase to transcribe specifically from heat-shock promoters (2–4). The increase in σ^{32} level results from both increased synthesis and stabilization of normally unstable σ^{32} ($t_{1/2}$ = 1 min), whereas the subsequent decrease in σ^{32} has been thought to depend on shutoff of synthesis and destabilization of σ^{32} by the DnaK–DnaJ chaperone-mediated autogenous negative control (5–9). The free pool of DnaK and/or DnaJ was thought to act as a cellular thermometer that modulates expression of all HSPs by monitoring the state of protein folding (9–11).

Heat-induced stabilization of σ^{32} occurs rapidly although transiently. The half-life of σ^{32} increases from 1 to 8 min for the first several minutes, and returns to about 1 min by 10 min after shift (7–9). The initial stabilization probably results from sequestering σ^{32} away from the DnaK–DnaJ chaperones because of heat-induced accumulation of unfolded or misfolded proteins and facilitating σ^{32} to bind core RNA polymerase, whereas subsequent destabilization results from accumulation of DnaK–DnaJ chaperones and proteases caused by the increase in σ^{32} (9–11). Accumulation of abnormal proteins without temperature upshift induces HSP synthesis (12) through stabilization but not induced synthesis of σ^{32} (13, 14). Although the membrane-bound ATP-dependent metalloprotease FtsH (HflB) was first shown to be responsible for rapid turnover of σ^{32} (15, 16), a set of cytosolic proteases including HslVU (ClpYQ) also participate in degradation of σ^{32} *in vivo* and *in vitro* (17, 18). The bulk of heat-shock proteases may therefore collectively serve to modulate the heat-shock response as well as degrade much of misfolded or abnormal proteins to cope with heat and other stresses.

Heat-induced synthesis of σ^{32} occurs primarily at the level of translation and is independent of the DnaK–DnaJ chaperone functions (8, 9, 19, 20). Mutational analyses of expression of *rpoH–lacZ* gene fusion combined with *in vitro* structural probing of *rpoH* mRNA established the importance of secondary structure (with appropriate stability) of the 5′ portion (+1 to 230 nt) of mRNA formed between the translation initiation region and part of the internal region for thermoregulation (20–22). Temperature-melting profiles of RNA segments (−60 to + 247 nt) with or without mutation(s) revealed an inverse correlation between thermostability and expression *in vivo*. Moreover, toeprint analyses with a synthetic mRNA fragment, purified 30S ribosome, and $tRNA_f^{Met}$ revealed a strong correlation between the formation of mRNA-30S ribosome-$tRNA_f^{Met}$ ternary complex *in vitro* and expression *in vivo* at different temperatures (23). These results led us to propose that the *rpoH* mRNA alone, with no additional regulatory factors, acts as a thermosensor in the translational control of σ^{32}. However, a major question remained concerning the nature of shutoff of σ^{32} synthesis during the adaptation phase.

Early observation of shutoff of σ^{32} synthesis even under control of the λP_L promoter suggested that it occurs posttranscriptionally (6). *E. coli* mutants deficient in the *dnaK–dnaJ* chaperones showed both defective shutoff of synthesis and degradation of σ^{32}, suggesting negative control mediated by the chaperones (8, 9). Analyses of the *rpoH–lacZ* gene fusion suggested involvement of an internal region of σ^{32} (region C; 122–144 aa) in the control of both DnaK–DnaJ-mediated shutoff of synthesis and stability of the σ^{32}–β-galactosidase fusion protein (24), although core RNA polymerase binding rather than DnaK binding or σ^{32} stability was recently shown to be affected by mutations in this region (25, 26). In any event, shutoff of σ^{32} synthesis could not be separated from the control of σ^{32} stability, despite the clear distinction between controls of heat-induced synthesis and stability of σ^{32} (3, 4, 27). In addition, the changing stability of σ^{32} at different phases prevented accurate determination of synthesis rates during the heat-shock response.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviation: HSP, heat shock protein.

*Present address: Graduate School of Biosciences, Nara Institute of Science and Technology, Ikoma 630-0101, Japan.

†To whom reprint requests should be addressed. E-mail: tyura@hsp.co.jp.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.080495197. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.080495197

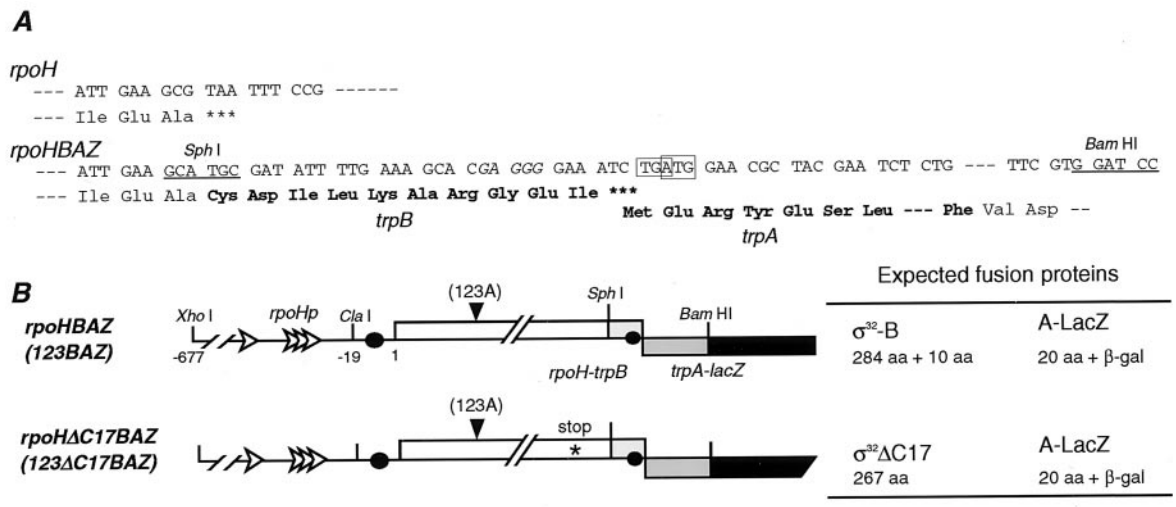

Fig. 1. (*A*) Sequences of the 3′ end of *rpoH* (*Upper*) and the *trpBA* junction of *rpoHBAZ* (*Lower*). In the *rpoHBAZ* construct, the C-terminal Ala codon of *rpoH* was fused in-frame with the 5′ end of *trpB* at the *Sph*I site, whereas the 5′ end of *trpA* is fused in-frame to codon 9 of *lacZ* at the *Bam*HI site. The stop codon (TGA) of *trpB* and the initiation codon (ATG) of *trpA* are boxed. Amino acids in bold letters originate from the *trpBA* junction. (*B*) Schematic diagrams of *rpoHBAZ* and its derivatives constructed as described in *Materials and Methods*. Arrowheads indicate the position of the G123A mutation that enhances translation, and parentheses indicate the mutation or constructs containing the mutation. The expected fusion protein products for each construct are indicated to the right.

We now show that the heat-induced synthesis of σ^{32} does not shut off at the translation level by using a reporter system without the complication of σ^{32} stability change. In addition, σ^{32} was shown to be destabilized to an extent much greater than had previously been thought. The control of the σ^{32} level during the heat-shock response appears to rest on an intricate balance between the efficiency of *rpoH* mRNA translation primarily determined by ambient temperature and the rate of σ^{32} turnover modulated by the chaperone (and protease)-mediated negative control.

Materials and Methods

Bacteria, Phage, and Growth Media. *E. coli* K-12, strains MC4100 [*araD* Δ*(argF-lac)U169 rpsL relA flbB deoC ptsF rbsR*] (28) and MG1655 (prototroph) were used for most experiments. Strain KY1603 (Δ*rpoH30*::*kan zhf50*::Tn*10 suhX401*), which lacks σ^{32} and overproduces GroEL–GroES chaperones (29), was used to examine σ^{32}-like function encoded by λ*rpoHBAZ* and its derivatives. λTLF97–3 vector (30) was used to construct *rpoH–lacZ* gene fusions. Synthetic medium M9 (31) supplemented with 0.2% glucose, thiamine (2 μg/ml), and all amino acids except for Met (20 μg/ml each) was used for pulse-labeling experiments. MacConkey lactose agar (Difco) and L agar containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) (30 μg/ml) were used for isolation of lysogens carrying λ*rpoHBAZ* prophage.

Construction of a *rpoH–lacZ* Fusion with the *trpBA* Junction. The entire *rpoH* gene including promoters and 3′ untranslated region was inserted into the pACYC177 vector between the *Xho*I and *Bam*HI sites. The C-terminal (Ala) and termination (UAA) codons of *rpoH* were replaced by a *Sph*I site without changing the Ala residue by using PCR site-directed mutagenesis (Fig. 1). A DNA fragment containing the *trpBA* junction was obtained by PCR with pMS436S (32) as a template and 5′ and 3′ primers that correspond to the respective ends of the fragment with some protruding extra nucleotides to create the *Sph*I or *Bam*HI site, respectively. The resulting *Sph*I–*Bam*HI fragment was then substituted for the *Sph*I–*Bam*HI segment on pACYC177. The *Xho*I–*Bam*HI fragment cut out from the latter plasmid was inserted into λTLF97–3 vector to yield an in-frame fusion with *lacZ* (λ*rpoHBAZ*). A derivative of *rpoHBAZ* carrying the G123A mutation was obtained by replacing the *rpoH* gene on pACYC177 by the corresponding region of pFRP103 containing the mutation (21). To construct *rpoHΔC17BAZ* and *123ΔC17BAZ*, the 17th codon from the C terminus of *rpoH* was converted to UGA by PCR mutagenesis and substituted for *rpoH* on pACYC177. Nucleotide sequences of all pertinent regions were confirmed by DNA sequencing. The resulting gene fusions were transferred to λTLF97–3 by *in vitro* packaging, introduced into strain MC4100, and confirmed for monolysogeny by PCR (33).

Determination of Synthesis Rates and Stability of σ^{32} and Fusion Proteins. Mid-logarithmic-phase cells were labeled with L-[^{35}S]Met (600 or 1,200 Ci/mmol; 100 or 200 μCi/ml) for 15–60 s with or without chase with unlabeled Met (200 μg/ml) to determine synthesis rates or stability as indicated for each set of experiments. Portions of labeled cells were treated with 5% trichloroacetic acid, and the resulting precipitates were washed with acetone and suspended in buffer containing SDS. Samples with equal radioactivities were mixed with a fixed amount of CAG11033 cell extract containing a truncated form of σ^{32} (7) or JM103 cell extract containing β-galactosidase ω protein (both labeled with [^{35}S]Met), and treated with antibody against σ^{32} or β-galactosidase (Organon Teknika–Cappel) for determining synthesis rates of σ^{32} or LacZ fusion proteins, respectively. Immunoprecipitates were subjected to SDS/PAGE essentially as described (22). Intensities of radioactive protein bands were quantified with a FUJIX (Tokyo) BAS2000 Imaging Analyzer to determine synthesis rates or stability after correction for their recovery by using truncated σ^{32} or ω as a reference.

β-Galactosidase Activity. Cells were grown at 30°C in M9 medium and assayed for β-galactosidase activity by the standard procedure (34).

Recombinant DNA and Other General Techniques. These were performed essentially as described by Sambrook *et al.* (35) and Miller (34).

Results

Translational Coupling Between σ^{32} and LacZ: Analysis of Shutoff of Synthesis Independent of Degradation. To analyze the possible translational shutoff of σ^{32} without complications arising from

Table 1. Expression of A-LacZ fusion protein from MC4100 (λ*rpoHBAZ*) and its derivatives

Construct	β-gal activity of A-LacZ, Miller U
rpoHBAZ	222.93 ± 10.10
rpoHΔC17BAZ	82.36 ± 2.40
123BAZ	762.25 ± 25.78
123ΔC17BAZ	81.77 ± 2.21

Cells were grown in M9 medium at 30°C to midlog phase and assayed for β-galactosidase activity.

change in σ^{32} stability during the heat-shock response, we constructed a reporter system by using translational coupling that occurs at the *trpB–trpA* junction, in which translation of the downstream gene (*trpA*) depends largely on complete translation of upstream *trpB* (36). The entire *rpoH* gene including promoters but omitting the stop codon (UAA) was fused with the 92-bp *trpBA* junction and with *lacZ* (Fig. 1*A*; *rpoHBAZ*). This generated two fusion genes encoding σ^{32} with the C-terminal 10 aa of TrpB (σ^{32}-B) and LacZ with the N-terminal 20 aa of TrpA (A-LacZ) (Fig. 1*B*). The *trpBA* junction containing a direct overlap between the *trpB* stop codon (UG<u>A</u>) and the *trpA* initiation codon (<u>A</u>UG) was expected to couple translation of upstream σ^{32}-B with that of downstream A-LacZ. The fusion was constructed on a single copy vector λTLF97–3 (30) and was inserted into the chromosome of strain MC4100, resulting in a lysogen that carries the λ*rpoHBAZ* prophage.

To test the validity of this system, three mutant derivatives were constructed: the *123BAZ* fusion exhibiting high-translation efficiency caused by the G123A mutation (of *rpoH*) which disrupts the mRNA secondary structure (21), and a pair of fusions that carry an extra stop codon (UGA) at the 17th codon from the C terminus of *rpoH* with or without G123A (*rpoHΔC17BAZ* and *123ΔC17BAZ*) (Fig. 1*B*). When expression of A-LacZ from these constructs was determined by measuring β-galactosidase activity, expression from *123BAZ* was 3.4-fold higher than that from the control (*rpoHBAZ*), as expected (Table 1). Moreover, expression from the two constructs carrying an extra stop codon was equally low, indicating that A-LacZ expression was markedly reduced independent of the efficiency of upstream translation. The low but significant activities observed with the latter constructs represent intrinsic *trpA–lacZ* translation independent of translation from upstream. These results showed effective translational coupling between the two fusion genes.

We also confirmed that the activity and expression pattern of σ^{32}-B is similar to those of authentic σ^{32}. When λ*rpoHBAZ* was introduced into strain KY1603 (Δ*rpoH* strain that overproduces GroE proteins) unable to grow at above 40°C (29), the resulting lysogen regained the ability to grow at 42°C (data not shown). In contrast, λ*rpoHΔC17BAZ* was inactive in this respect, indicating that σ^{32}-B but not the C-terminal truncated derivative is functionally active and promotes transcription from the heat-shock promoters. Furthermore, when the λ*rpoHBAZ* lysogen of wild-type MC4100 grown at 30°C was shifted to 42°C, synthesis of σ^{32}-B as well as authentic σ^{32} encoded by the chromosomal *rpoH* was appreciably enhanced and shut off after about 3 min, as expected (Fig. 2*A*). The stability of σ^{32}-B and σ^{32}ΔC17 was also tested and found to be similar to that of authentic σ^{32} (Fig. 4 *C* and *D*).

Heat-Induced Translation of σ^{32} Does Not Shut Off During the Adaptation Phase. By using the above reporter system, we asked whether heat-induced synthesis of σ^{32} is shut off at the level of translation. If shutoff occurs at the translational level, expression of A-LacZ from *rpoHBAZ* should be heat induced and shut off,

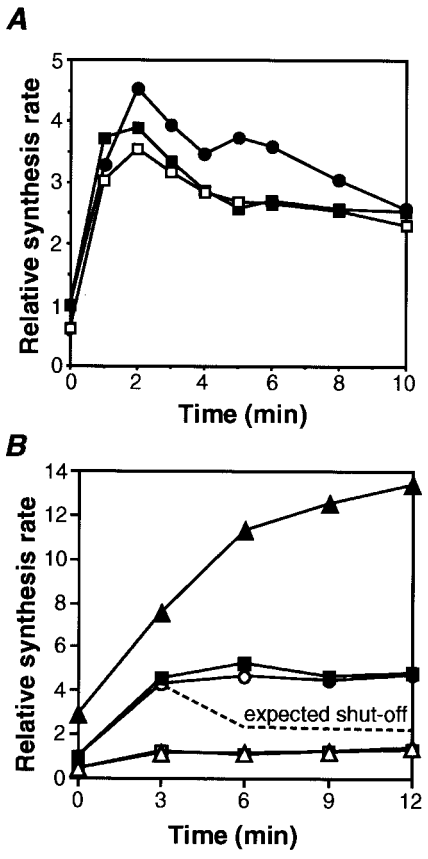

Fig. 2. Heat-induced synthesis of σ^{32}, σ^{32}-B, and A-LacZ in strain MC4100 carrying λ*rpoHBAZ* or its derivative. (*A*) Cells were grown in minimal medium at 30°C and shifted to 42°C at $t = 0$. Samples were taken at the times indicated and pulse labeled with [^{35}S]Met (1,200 Ci/mmol; 100 μCi/ml) for 30 s. The labeled σ^{32} and σ^{32}-B were precipitated by σ^{32}-specific antiserum and analyzed by SDS/PAGE followed by quantification as described in *Materials and Methods*. Values were normalized to the $t = 0$ value for each protein and then to σ^{32} in MC4100. (●) σ^{32} in MC4100; (■) σ^{32} in MC4100 (λ*rpoHBAZ*); and (□) σ^{32}-B in MC4100 (λ*rpoHBAZ*). (*B*) Cells were grown and treated essentially as in *A*, except that the pulse labeling with [^{35}S]Met was done at 600 Ci/mmol for 60 s followed by chase with excess unlabeled Met for 60 s. The labeled A-LacZ was precipitated with anti-β-galactosidase antiserum and analyzed by SDS/PAGE. Quantification was done as in *A* and normalized to $t = 0$ for each protein and then to *rpoHBAZ*. The dotted line indicates the expected curve if the heat-induced synthesis from *rpoHBAZ* was shut off. (○) TLF247; (■) *rpoHBAZ*; (▲) *123BAZ*; (□) *rpoHΔC17BAZ*; and (△) *123ΔC17BAZ*.

like that observed with σ^{32}-B or authentic σ^{32} (Fig. 2*A*). Thus, synthesis rates of A-LacZ from *rpoHBAZ* and its mutant derivatives were determined after temperature upshift. Contrary to the expectation, synthesis of A-LacZ was normally heat induced but did not shut off (Fig. 2*B*), like the *rpoH–lacZ* fusion TLF247 constructed (22) and thought to be unable to shut off because of the lack of region C (24). Moreover, the expression of A-LacZ from *123BAZ* was about threefold higher than that from the control at 30°C and further enhanced on shift to 42°C as expected, but again failed to shut off.

In contrast, A-LacZ expression from *rpoHΔC17BAZ* or *123ΔC17BAZ* carrying an extra stop codon was very low at 30°C and only slightly enhanced on shift to 42°C, consistent with the lack of translational coupling (Fig. 2*B*). This also showed that heat induction of A-LacZ observed with *rpoHBAZ* occurs as the result of translational coupling and not independent of upstream translation. These results strongly suggested that shutoff does not take place at the translational level. The apparent shutoff of

synthesis of σ^{32} or σ^{32}-LacZ fusion observed (7, 20, 24) therefore most probably reflects posttranslational events occurring during the adaptation phase.

Absence of Apparent Shutoff with Shorter Pulse Labeling. Because of the very low content of σ^{32}, most previous work used 1-min pulse labeling with [^{35}S]Met to measure the synthesis rates. However, it was difficult to follow the change in synthesis rates accurately because of the intrinsic instability. We therefore reexamined σ^{32} synthesis by using shorter pulse-labeling protocols with strain MC4100. It was anticipated that apparent synthesis rates obtained even with 30-s pulse can represent only approximations. In agreement with the results presented above, apparent shutoff was observed with a 60-s pulse but less clearly with a 30-s pulse (Fig. 3 *A* and *B*). Most significantly, when the 15-s pulse was used, little or no apparent shutoff occurred even after a 10-min incubation.

It should be noted that the initial heat induction seen for 2–3 min was essentially identical in all cases, and differential responses were found only after 4–5 min during the adaptation phase, by which time σ^{32} was known to be destabilized. Experiments with another prototrophic strain (MG1655) gave similar results, although the apparent shutoff with a 60-s pulse was slightly less than that with MC4100 (Fig. 3 *C* and *D*). These results unambiguously demonstrated the lack of shutoff of heat-induced σ^{32} synthesis and suggested that the apparent shutoff observed with longer pulse may result from much severer destabilization of σ^{32} than had previously been suspected.

Extreme Instability of σ^{32} During Adaptation Phase May Account for Apparent Shutoff of Synthesis. Consistent with the above findings, the apparent synthesis rate of σ^{32} in cells pulse labeled at 10 min after temperature upshift was only about twofold higher than that at 30°C (t = 0) when the results with 60-s pulse were compared (Fig. 3), in contrast to the four- to fivefold heat induction of stable σ^{32}-β-galactosidase fusion observed with TLF247 (22). This suggested that stability of σ^{32} during the adaptation phase may be lower than at 30°C. Indeed, recent results revealed that the half-life of σ^{32} in cells steadily growing at 42°C is much shorter (≈15 s) than at 30°C (1 min) with strain MG1655 (18). We therefore examined whether such a drastic destabilization of σ^{32} occurs within 10 min after temperature upshift.

Cells of MC4100 were pulse labeled for 30 s before (30°C) and 10 min after shift to 42°C, and chased with excess unlabeled Met at the respective temperatures. Based on these experiments, the half-life of σ^{32} at 30°C and 10 min after shift to 42°C was estimated to be 65 and 20 s, respectively (Fig. 4 *A* and *B*). Quite similar results were obtained with strain MG1655 (data not shown). These results indicated that stability of σ^{32} changes drastically and dynamically during the heat-shock response, as predicted from the above experiments on σ^{32} synthesis. Thus, normally unstable σ^{32} at 30°C ($t_{1/2}$ = 1 min) is stabilized almost immediately for 4–5 min ($t_{1/2}$ = 8 min) on shift to 42°C (8), followed by rapid destabilization leading to an extreme instability ($t_{1/2}$ = 20 s) within 10 min. Apparent shutoff of synthesis of σ^{32} observed with a 60-s pulse may therefore be explained primarily by extreme instability of σ^{32} that presumably counterbalances the excessive synthesis of σ^{32} that would arise from continuously enhanced translation.

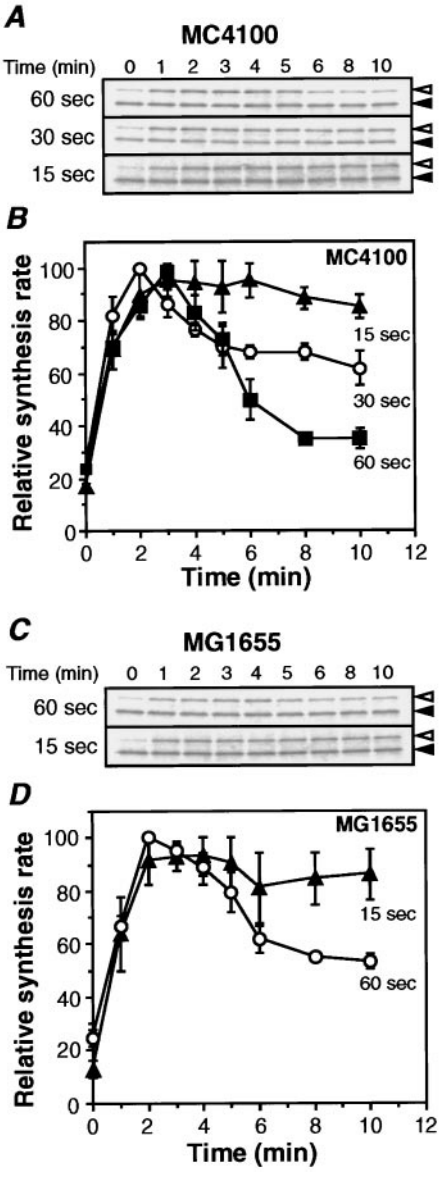

Fig. 3. Effects of varying length of pulse labeling on σ^{32} synthesis on heat shock. (*A* and *C*) SDS/PAGE patterns of σ^{32} synthesized in MC4100 or MG1655. Cells were grown in minimal medium at 30°C, shifted to 42°C, and samples taken at various points were pulse labeled for the indicated period with [^{35}S]Met (600 Ci/mmol, 100 μCi/ml for 60-s pulse; 1,200 Ci/mmol, 100 μCi/ml for 30- or 15-s pulse). (◁) The labeled σ^{32} was precipitated and analyzed by SDS/PAGE as in Fig. 2*A*. (◀) Reference protein to correct for sample loss. (*B* and *D*) Quantitation of relative synthesis rates. The band intensities were quantified and normalized to the maximum value (set as 100) for each experiment and shown as relative synthesis rates. Average values of at least three independent experiments are presented with standard errors.

Discussion

By using the reporter system involving translational coupling and the very short pulse-labeling protocols, we demonstrated that the apparent shutoff of heat-induced σ^{32} synthesis does not result from translational repression, contrary to what had previously been believed. Instead, σ^{32} was found to become extremely unstable ($t_{1/2}$ = 20 s) within 10 min after temperature shift (Fig. 4), consistent with the instability observed with strain MG1655 under steady-state growth at 42°C ($t_{1/2}$=10–15 s; ref. 18). The apparent shutoff of σ^{32} synthesis during the adaptation phase can therefore be explained primarily by severe instability of σ^{32} at high temperature. Whereas the early work established the basic regulatory features including initial stabilization of σ^{32} followed by destabilization, the extent of destabilization was underestimated and thought to be comparable to that at 30°C ($t_{1/2}$ = 1 min; ref. 7). The unusual instability together with the changing stability precluded accurate measurement of synthesis rates of σ^{32} by 60-s pulse labeling.

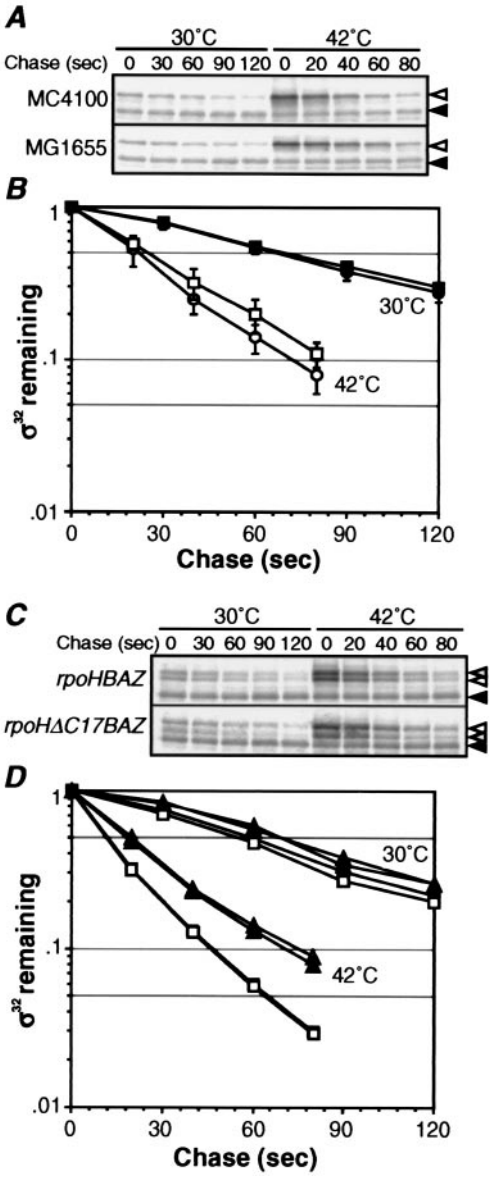

Fig. 4. Differential stability of σ^{32} at 30°C and 10 min after shift to 42°C. (*A* and *C*) SDS/PAGE patterns of σ^{32} remaining in pulse-chase experiments. Cells were grown at 30°C, shifted to 42°C, and portions taken at $t = 0$ and 10 min were pulse labeled with [^{35}S]Met (1,200 Ci/mmol, 200 μCi/ml) for 30 s, and chased with excess unlabeled Met for 30 or 20 s at 30 or 42°C, respectively, and set as $t = 0$. Aliquots were then taken at the times indicated, and σ^{32} (◁) and σ^{32}-B or $\sigma^{32}\Delta$C17 (hatched arrowheads) were immunoprecipitated and analyzed by SDS/PAGE as in Fig. 2*A*. (◀) Reference as in Fig. 2. (*B* and *D*) Quantification of protein stability. (*B*) ●, MC4100, 30°C; ○, MC4100, 42°C; ■, MG1655, 30°C; and □, MG1655, 42°C. (*D*) □, σ^{32}-B in MC4100 (λ*rpoHBAZ*); ■, σ^{32} in MC4100 (λ*rpoHBAZ*); △, $\sigma^{32}\Delta$C17 in MC4100 (λ*rpoHΔC17BAZ*); and ▲, chromosomally encoded σ^{32} in MC4100 (λ*rpoHΔC17BAZ*).

The above conclusion is consistent with the previous failure to separate shutoff of synthesis and degradation when involvement of specific segments of σ^{32} or transacting factors in the two processes were assessed (9, 24). However, some results seemed to be an apparent contradiction. First, heat-induced synthesis of σ^{32} in the *dnaJ259* mutant failed to shut off (60-s pulse) despite the fact that σ^{32} synthesized 3 min after shift to 42°C was as unstable as the wild type (9). When we examined σ^{32} stability during the adaptation phase with the *dnaJ259* mutant of MC4100, the half-life of σ^{32} was about 1 min, significantly longer than that for the wild type, indicating marked stabilization (data not shown). However, this difference in half-life may not be sufficient to explain the difference in the shutoff profile reported (9). Second, heat-induced synthesis of σ^{32} in the Δ*ftsH* mutant containing a suppressor (Δ*shfC*) seemed to shut off normally (60-s pulse) even though σ^{32} was markedly stabilized (37). We confirmed that σ^{32} synthesis with the same Δ*ftsH* Δ*shfC* mutant exhibits gradual apparent shutoff, although not as striking as that reported previously (data not shown). It should also be noted that this mutant shows very slow growth (by about threefold). Besides, we found that apparent shutoff hardly occurs with the Δ*shfC* control, as also seen in the reported results (37). Thus, significance of the apparent shutoff observed specifically with the Δ*ftsH* mutant remains unclear.

The inability of $\sigma^{32}\Delta$C17 to complement the temperature-sensitive growth of Δ*rpoH* strain (KY1603) agrees with the recent report on σ^{32} lacking the C-terminal 15 residues (38). When we measured the stability of σ^{32}-B and $\sigma^{32}\Delta$C17, they both exhibited stability very similar to that of authentic σ^{32}, the half-life in the λ*rpoHBAZ* lysogen of MC4100 before or after temperature upshift being similar to, or slightly shorter than, that of σ^{32} in MC4100 (Fig. 4 *C* and *D*). The results with $\sigma^{32}\Delta$C17 was unexpected, because it was recently reported that σ^{32} with the C-terminal 15 aa replaced by 6 unrelated residues (σ^{32}CΔ) expressed in strain KY1603 (Δ*rpoH suhX401*) was quite stable at 30°C (38). The apparent discrepancy could be caused by the extra amino acids added during construction of the expression plasmid or the different host bacteria used (Δ*rpoH* vs. *rpoH*$^+$).

To sum up, HSP synthesis in *E. coli* is primarily regulated by the dynamic interplay between two antagonistic pathways affecting synthesis and degradation of σ^{32}. One pathway controls *rpoH* translation by temperature-directed melting of the mRNA secondary structure (22, 23) in which the mRNA serves not only as a messenger but as a thermosensor and regulator of translation. Such a multifunctional mRNA must provide a unique and sensitive means of responding very rapidly to sudden changes in ambient temperature. However, this is a steady state rather than transient response to high temperature. In contrast, the other pathway controls degradation of σ^{32} as mediated by the DnaK–DnaJ chaperones and ATP-dependent heat-shock proteases which should serve to monitor the cellular state of protein folding, thereby fine-tuning the level of σ^{32} to cope with constant changes in cellular requirements. The DnaK–DnaJ chaperones can also modulate HSP synthesis by inhibiting σ^{32} activity (37, 39). Recent work suggested that σ^{32} itself directly responds to high temperature by changing its susceptibility to proteases (18). The combined results therefore indicate that regulation of the σ^{32} level during the heat-shock response rests on intricate balance between elevated synthesis and elevated turnover of σ^{32} that are controlled by distinct but interconnected pathways. The dynamic role played by the chaperones and proteases in modulating the stress response in this and other systems remains an outstanding issue for future investigation.

We thank M. Nakayama for technical assistance and M. Tanaka for assistance in preparing figures.

1. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. (1994) *The Biology of Heat Shock Proteins and Molecular Chaperones* (Cold Spring Harbor Lab. Press, Plainview, NY).
2. Gross, C. A., Straus, D. B., Erickson, J. W. & Yura, C. (1990) in *Stress Proteins in Biology and Medicine*, eds. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 167–189.
3. Gross, C. A. (1996) in *Escherichia coli and Salmonella: Cellular and Molecular Biology*, eds. Neidhardt, F. C., Curtiss, R., III, Ingraham, J. L., Lin, E. C. C., Low, K. B., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M. & Umbarger, H. E. (ASM Press, Washington, DC), pp. 1389–1399.

4. Yura, T., Nagai, H. & Mori, H. (1993) *Annu. Rev. Microbiol.* **47,** 321–350.
5. Tilly, K., McKittrick, N., Zylicz, M. & Georgopoulos, C. (1983) *Cell* **34,** 641–646.
6. Grossman, A. D., Straus, D. B., Walter, W. A. & Gross, C. A. (1987) *Genes Dev.* **1,** 179–184.
7. Straus, D., Walter, W. & Gross, C. A. (1987) *Nature (London)* **329,** 348–351.
8. Tilly, K., Spence, J. & Georgopoulos, C. (1989) *J. Bacteriol.* **171,** 1585–1589.
9. Straus, D., Walter, W. & Gross, C. A. (1990) *Genes Dev.* **4,** 2202–2209.
10. Craig, E. A. & Gross, C. A. (1991) *Trends Biochem. Sci.* **16,** 135–140.
11. Bukau, B. (1993) *Mol. Microbiol.* **9,** 671–680.
12. Goff, S. A. & Goldberg, A. L. (1985) *Cell* **41,** 587–595.
13. Wild, J., Walter, W. A., Gross, C. A. & Altman, E. (1993) *J. Bacteriol.* **175,** 3992–3997.
14. Kanemori, M., Mori, H. & Yura, T. (1994) *J. Bacteriol.* **176,** 5648–5653.
15. Herman, C., Thevenet, D., D'Ari, R. & Bouloc, P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 3516–3520.
16. Tomoyasu, T., Gamer, J., Bukau, B., Kanemori, M., Mori, H., Rutman, A. J., Oppenheim, A. B., Yura, T., Yamanaka, K., Niki, H., *et al.* (1995) *EMBO J.* **14,** 2551–2560.
17. Kanemori, M., Nishihara, K., Yanagi, H. & Yura, T. (1997) *J. Bacteriol.* **179,** 7219–7225.
18. Kanemori, M., Yanagi, H. & Yura, T. (1999) *J. Biol. Chem.* **274,** 22002–22007.
19. Kamath-Loeb, A. S. & Gross, C. A. (1991) *J. Bacteriol.* **173,** 3904–3906.
20. Nagai, H., Yuzawa, H. & Yura, T. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 10515–10519.
21. Yuzawa, H., Nagai, H., Mori, H. & Yura, T. (1993) *Nucleic Acids Res.* **21,** 5449–5455.
22. Morita, M., Kanemori, M., Yanagi, H. & Yura, T. (1999) *J. Bacteriol.* **181,** 401–410.
23. Morita, T. M., Tanaka, Y., Kodama, T., Kyogoku, Y., Yanagi, H. & Yura, T. (1999) *Genes Dev.* **13,** 655–665.
24. Nagai, H., Yuzawa, H., Kanemori, M. & Yura, T. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 10280–10284.
25. Joo, D. M., Nolte, A., Calendar, R., Zhou, Y.-N. & Jin, D. J. (1998) *J. Bacteriol.* **180,** 1095–1102.
26. Arsene, F., Tomoyasu, T., Mogk, A., Schirra, C., Schulze-Specking, A. & Bukau, B. (1999) *J. Bacteriol.* **181,** 3552–3561.
27. Yura, T. (1996) *Genes Cells* **1,** 277–284.
28. Casadaban, M. (1976) *J. Mol. Biol.* **104,** 541–555.
29. Kusukawa, N. & Yura, T. (1988) *Genes Dev.* **2,** 874–882.
30. St. Pierre, R. & Linn, T. (1996) *Gene* **169,** 65–68.
31. Nagai, H., Yano, R., Erickson, J. W. & Yura, T. (1990) *J. Bacteriol.* **172,** 2710–2715.
32. Hirano, M., Shigesada, K. & Imai, M. (1987) *Gene* **57,** 89–99.
33. Powel, B. S., Court, D. L., Nakamura, Y., Rivas, M. P. & Turnbough, C. L., Jr. (1994) *Nucleic Acids Res.* **22,** 5765–5766.
34. Miller, J. (1992) *A Short Course in Bacterial Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
35. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
36. Das, A. & Yanofsky, C. (1984) *Nucleic Acids Res.* **12,** 4757–4768.
37. Tatsuta, T., Tomoyasu, T., Bukau, B., Kitagawa, M., Mori, H., Karata, K. & Ogura, T. (1998) *Mol. Microbiol.* **30,** 583–593.
38. Blaszczak, A., Georgopoulos, C. & Liberek, K. (1999) *Mol. Microbiol.* **31,** 157–166.
39. Tomoyasu, T., Ogura, T., Tatsuta, T. & Bukau, B. (1998) *Mol. Microbiol.* **30,** 567–581.