Marked Instability of the σ32 Heat Shock Transcription Factor at High Temperature

IMPLICATIONS FOR HEAT SHOCK REGULATION*

(Received for publication, April 12, 1999, and in revised form, May 17, 1999)

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The heat shock response in *Escherichia coli* depends on a transient increase in the intracellular level of σ32 that results from both increased synthesis and transient stabilization of normally unstable σ32. Although the membrane-bound ATP-dependent protease FtsH (HflB) plays an important role in degradation of σ32, our previous results suggested that several cytosolic ATP-dependent proteases including HslVU (ClpQY) are also involved in σ32 degradation (Kanemori, M., Nishihara, K., Yanagi, H., and Yura, T. (1997) J. Bacteriol. 179, 7219–7225). We now report on the ATP-dependent proteolysis of σ32 by purified HslVU protease and its unusual dependence on high temperature: σ32 was rapidly degraded at 44 °C, but with much slower rates (~15-fold) at 35 °C. FtsH-dependent degradation of σ32 also gave similar results. In agreement with these results in *vitro*, the turnover of σ32 in normally growing cells at high temperature (42 °C) was much faster than at low temperature (30 °C). Taken together with other evidence, these results suggest that the σ32 level during normal growth is primarily determined by the stability (susceptibility to proteases) and synthesis rate of σ32 set by ambient temperature, whereas fine adjustment such as transient stabilization of σ32 observed upon heat shock is brought about through monitoring changes in the cellular state of protein folding.

When cells and organisms are exposed to high temperature, synthesis of a set of heat shock proteins is rapidly induced. The induction generally occurs at the transcriptional level mediated by specific transcription factors. In *Escherichia coli*, the level of the heat shock transcription factor σ32 (encoded by the *rpoH* gene) rapidly and transiently increases upon temperature upshift and directs RNA polymerase to transcribe the heat shock genes encoding molecular chaperones (GroEL, DnaK, etc.), ATP-dependent proteases (Lon (La), ClpX, HslVU (ClpQY), and FtsH (HflB)), and other heat shock proteins (1–4). The increase in the σ32 level depends on both increased synthesis and stabilization of normally unstable σ32 (half-life of ~1 min) (5). Production of abnormal proteins such as those containing amino acid analogs or heterologous proteins can also induce heat shock proteins and mimic the heat shock response (6–8). However, in the latter cases, the σ32 level increases as the result of stabilization and not of increased synthesis of σ32 (9, 10). Thus, stabilization and enhanced synthesis of σ32 observed upon temperature upshift represent two distinct events that presumably involve different signaling pathways (3, 4).

Upon temperature upshift from 30 to 42 °C, the rate of σ32 synthesis increases ~10-fold within 3–4 min (5). The induction occurs at the translational level mediated by the secondary structure of the 5'-portion of *rpoH* mRNA (11–13). Recent work showed that high temperature directly disrupts the mRNA secondary structure, perhaps without involvement of cellular factors, leading to enhanced ribosome entry and initiation of translation (14). Besides translational induction, marked stabilization of σ32 (8-fold) occurs for the first 4–5 min upon heat shock, followed by rapid destabilization (5). The DnaK chaperone one (DnaK, DnaJ, and GrpE) is required for the rapid σ32 degradation because σ32 is markedly stabilized in *dnaK*/*dnaJ*/*grpE* mutants (15, 16). Although the exact roles of these chaperones in σ32 turnover remain unknown, transient stabilization of σ32 has been thought to occur by titrating the chaperones away from σ32 by unfolded proteins accumulating at high temperature since these chaperones are also involved in dealing with unfolded or misfolded proteins (17, 18). Accordingly, free DnaK and/or DnaJ chaperones that can engage in the turnover of σ32 have been proposed to act as a “cellular thermometer” that should monitor changes in the folding state of the cell (19, 20). In agreement with these proposals, DnaK/DnaJ chaperones were shown to be normally limiting in *vivo*: a slight increase or decrease in the chaperones causes a decrease or increase in σ32 (and consequently, heat shock proteins), respectively (21). A membrane-bound ATP-dependent metalloprotease (FtsH) was the first protease shown to degrade σ32 *in vivo* and *in vitro* (22, 23). More recent data suggested that a cytosolic ATP-dependent protease (HslVU) along with other proteases (ClpAP (Ti) and Lon) can also participate in the turnover of σ32 *in vivo* (24). HslVU is a two-component ATP-dependent protease consisting of a catalytic subunit (homododecamer of HslV (ClpQ)) and an ATPase subunit (homohexa- or heptamer of HsIC (ClpY)); one HslV complex is flanked by HslU complexes to form structures resembling the ClpAP protease and the eukaryotic 26 S proteasome (25, 26).

In this report, we examined degradation of σ32 *in vitro* by the HslVU protease and found that purified HslVU can degrade σ32 in an ATP-dependent manner. Furthermore, the susceptibility of σ32 to HslVU- or FtsH-mediated proteolysis *in vitro* markedly increased at high temperature. The marked instability of σ32 at high temperature was also demonstrated *in vivo*, suggesting that the susceptibility of σ32 to proteases reflecting ambient temperature plays a critical role in the regulation of σ32 and the heat shock response in *E. coli*.

**EXPERIMENTAL PROCEDURES**

*Bacterial Strains and Plasmids*—MG1655 (prototrophic *E. coli* K12) was used for purification of σ32 and for most experiments *in vivo*, and KY2691 (MG1655 ΔhslU (ΔclpPX-lon) ΔftsZ2691) (27) was used for...
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purification of HslV, HusU, and MBP-SulA fusion protein encoded by the respective plasmids. KY1603 lacking $\sigma^{32}$ but able to grow at 37 °C due to overproduction of GroE chaperones (MC4100 (F $\Delta araD \Delta argF-\lacI169$ rpsL relA $\Delta deoC$ ptsF $\Delta fisB$ aprE330 lacZ50/Trp1 $\Delta suC401$ (ApF3-PrpO-Dha-lacZ)) (28) was used for purification of RNA polymerase.

Plasmids pKTV1025 and pKTV1022 (27) carry the hslV and hslU genes, respectively, under control of the trc promoter on the pTrc99A vector (Amersham Pharmacia Biotech). A promoterless rpoH gene ($\Delta EcolR-NsiI$ fragment) was placed under control of the trc promoter on pTrc99A to obtain pKV1278. pMAL-c-sulA carrying a malE-sulA fusion gene was kindly donated by Y. Iishi and Y. Kato (Kyushu Institute of Technology). Other chemicals were obtained from Nacalai Tesque, Kyoto, or Wako Pure Chemicals (Osaka, Japan). Antiserum against FtsH was kindly supplied by Y. Akiyama (Kyoto University). Other proteins—All protein purification was carried out at 4 °C. HslV or HusU was purified from KY2691 cells harboring pKTV1025 or pKTV1022, respectively, as described (27). Purified FtsH was generously supplied by T. Obara (Kumamoto University).

$\sigma^{32}$ was purified from MG1655 cells harboring pKV1278 basically as described previously (30, 31). Cells were grown to mid-log phase in L broth containing ampicillin at 30 °C, and $\sigma^{32}$ synthesis was induced by IPTG. After 40 min, cells were harvested by centrifugation, resuspended in buffer A (50 mM Tris-HCl (pH 7.2), 2 mM EDTA, 1 mM dithiothreitol, 230 mM NaCl, 10% (v/v) sucrose, and 0.1% lysozyme) (30), and kept on ice for 30 min. After addition of sodium deoxycholate to 0.05%, cells were disrupted by sonication, and the resulting lysate was centrifuged at 75,000 × g for 90 min. The supernatant was treated with a column containing ammonium sulfate (0.18 g/ml), and the pellet was dissolved and dialyzed against buffer B (40 mM Hepes-KOH (pH 7.0), 0.1 mM EDTA, 1 mM dithiothreitol, 50% sodium sulfate, and 0.1% (v/v) glycerol) and loaded onto a HiPrep Sephacryl S-100 column (Amersham Pharmacia Biotech), and proteins were eluted with a linear gradient of NaCl.

The column was washed as described previously (31), and proteins were eluted with buffer C containing a linear gradient of NaCl. The fractions containing $\sigma^{32}$ were collected and stored at −70 °C.

RNA polymerase core enzyme consisting of $\beta$, $\beta'$, and two $\sigma$-subunits was purified from strain KY1603 lacking $\sigma^{32}$. Cells were grown to late-log phase in L broth at 37 °C, harvested by centrifugation, suspended in buffer A, and kept on ice for 30 min. After addition of sodium deoxycholate to 0.05%, cells were sonicated and centrifuged at 75,000 × g for 90 min, and proteins were precipitated by ammonium sulfate (0.35 g/ml). The pellet was resuspended and dialyzed against buffer B and loaded onto a HiLoad Q-Sepharose column (Amersham Pharmacia Biotech). Proteins were eluted with a linear gradient of NaCl, and the fractions containing RNA polymerase were precipitated by ammonium sulfate (0.35 g/ml). The pellet was resuspended and dialyzed against buffer C, and loaded onto a HiLoad Q-Sepharose column (Amersham Pharmacia Biotech). Proteins were eluted with a linear gradient of NaCl, and the fractions containing RNA polymerase were precipitated by ammonium sulfate (0.35 g/ml). The pellet was resuspended in buffer D and loaded onto a HiPrep Sephacryl S-300 column (Amersham Pharmacia Biotech), and RNA polymerase core enzyme was concentrated and stored at −70 °C.

MBP-SulA fusion protein was purified from KY2691 cells harboring pMAL-c-sulA. Cells were grown to mid-log phase in L broth containing ampicillin and production of MBP-SulA was induced by 0.84 mM IPTG. After 1 h of incubation, cells were harvested by centrifugation, resuspended in buffer E, and sonicated. MBP-SulA fusion protein was purified according to the instruction manual (New England Biolabs Inc.), except that buffer E was used as the column buffer. All purified proteins were >90% pure as estimated by SDS-PAGE followed by staining with Coomassie Brilliant Blue, and their concentration was determined by Bradford protein assays (Bio-Rad) (22).

**Enzymatic Assays**—The reaction mixture (50–120 µl) (33) for the HslVU protease contained 50 mM Tris-HCl (pH 8.0), 0.1 mM KCl, 1 mM dithiothreitol, 0.02% Triton X-100, 25 mM MgCl₂, 4 mM ATP, 16 µg/ml HslV, 40 µg/ml HusU, and 40 µg/ml substrates unless otherwise indicated. RNA polymerase (core enzyme) was added at a concentration of 480 µg/ml (1:1 RNA polymerase/substrate molar ratio). The reaction mixture (50–200 µl) contained 50 mM Tris acetate (pH 8.0), 5 mM magnesium acetate, 12.5 µM zinc acetate, 80 mM NaCl, 1.4 mM β-mercaptoethanol, 4 mM ATP, 40 µg/ml bovine serum albumin, and 40 µg/ml FtsH. The reaction was incubated at various temperatures, and the reaction was terminated by mixing with an equal volume of 2× sample buffer. Proteins were separated by SDS-PAGE and stained with Coomassie Brilliant Blue. Quantification of protein bands was done with a BioImage Intelligent Quantifier.

**Other Methods**—Nucleic acid manipulation (34), SDS-PAGE (29), and immunoblotting (24) were performed as described previously.

**RESULTS**

**ATP-dependent Degradation of $\sigma^{32}$ by the HslVU Protease**—Our previous data in vivo suggested that besides FtsH, the cytosolic ATP-dependent proteases including HslVU participate in the turnover of $\sigma^{32}$ (24). To test whether HslVU can directly degrade $\sigma^{32}$, we purified HslV and HusU separately from a pair of multiprotein-deficient KY2691 mutants lacking all known cytosolic ATP-dependent proteases, but harboring an expression plasmid for hslV or hslU. This permitted us to obtain HslV and HusU preparations with no contamination of other cytosolic ATP-dependent proteases. Although these strains carried the wild-type ftsH gene, no detectable amount of FtsH was found in either the HslV or HusU preparation used as judged by immunoblotting (data not shown). When purified $\sigma^{32}$ was incubated with HslV, HusU, and ATP under the standard assay conditions at 42 °C, marked degradation of $\sigma^{32}$ occurred, whereas no detectable degradation occurred when any of these components was omitted (Fig. 1A). These results support our previous observations in vivo, suggesting that HslVU and other cytoplasmic proteases directly contribute to the turnover of $\sigma^{32}$ to appreciable extents.

To determine the stoichiometry of HslV (19 kDa) and HusU (50 kDa) for the maximal proteolytic activity, the reaction was carried out by increasing the amount of HslV with a fixed amount of HusU (40 µg/ml, 0.8 µM protease) or by increasing the amount of HusU with a fixed amount of HslV (16 µg/ml, 0.84 µM protease). In both cases, the amount of $\sigma^{32}$ hydrolyzed increased with increasing amounts of the other component and reached the maximal activity when HslV and HusU were present at an approximate molar ratio of 1:1 proteases (Fig. 1B and C). These results are consistent with the notion that the HslVU protease consists of one HslV dodecamer and two HusU hexa- or heptamers (25, 26).

**Inhibition of $\sigma^{32}$ Degradation by RNA Polymerase (Core Enzyme)**—To examine the effect of RNA polymerase on $\sigma^{32}$ degradation by the HslVU protease, $\sigma^{32}$ was preincubated with RNA polymerase at 44 °C before adding HslVU. $\sigma^{32}$ was hardly degraded by HslVU under these conditions (Fig. 2, lane 5), whereas active degradation occurred in the control without RNA polymerase (lane 2), indicating that the prior interaction with RNA polymerase protected $\sigma^{32}$ from proteolytic attack by HslVU. By contrast, RNA polymerase did not affect degradation of the cell division inhibitor SulA, another substrate for HslVU (27, 35), fused to the maltose-binding protein (MBP-SulA) (lanes 8 and 11). When RNA polymerase was incubated with MBP-SulA fusion protein at 44 °C, about half of the RNA polymerase became insoluble and disappeared from the soluble fraction (lane 12). When preincubated with $\sigma^{32}$, however, most
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of the RNA polymerase remained soluble (lane 6), indicating that most of the $\sigma^{32}$ binds to the polymerase to form RNA polymerase holoenzyme.

Marked Temperature Dependence of $\sigma^{32}$ Degradation—When proteolysis of $\sigma^{32}$ by HslVU was examined at four different temperatures (35, 38, 41, and 44 °C), a striking temperature dependence was observed (Fig. 3 and Table I). Only limited degradation occurred at lower temperatures (35 and 38 °C), whereas rapid degradation was observed at higher temperatures (41 and 44 °C). The ratio of activities at 44 and 35 °C based on the initial rates of proteolysis was estimated to be ~15 (Table I). These results indicate that $\sigma^{32}$ can be degraded by HslVU at the physiological temperature range, but particularly efficiently at higher temperatures.

To address the question of whether $\sigma^{32}$ or HslVU is primarily responsible for the observed high temperature-dependent $\sigma^{32}$ degradation, two other substrates (MBP-SulA and $\alpha$-casein) were tested and compared with $\sigma^{32}$ for the temperature dependence of proteolysis. When MBP-SulA fusion protein was incubated with HslVU and ATP at various temperatures, only a weak temperature dependence was observed, unlike with $\sigma^{32}$: the fusion protein was degraded at 35 °C with appreciable efficiency, ~40% of that at 44 °C (Fig. 4A and Table I). Similar results were obtained when $\alpha$-casein was digested with HslVU (Fig. 4B and Table I). Taken together, these results suggest that $\sigma^{32}$ rather than HslVU is primarily responsible for the observed temperature dependence of proteolytic activities on high temperature.

FtsH Catalyzes the Highly Temperature-dependent Proteolysis of $\sigma^{32}$—To further confirm the unusual temperature dependence of $\sigma^{32}$ degradation, we carried out similar experiments with FtsH, known to degrade $\sigma^{32}$ in vivo and in vitro. Accordingly, $\sigma^{32}$ was incubated with FtsH at 35 or 44 °C in the presence of ATP under the conditions used by Tomoyasu et al. (23). As shown in Fig. 5A, $\sigma^{32}$ degradation was much faster (~4-fold) at 44 °C than at 35 °C. By contrast, $\alpha$-casein used as a control substrate was degraded with similar efficiencies at both temperatures (Fig. 5B), indicating that $\sigma^{32}$ is also susceptible to FtsH-mediated proteolysis particularly at high temperature. These results strongly suggest that the unusual high temperature dependence of $\sigma^{32}$ degradation is ascribed to certain structural features of $\sigma^{32}$, although the possibility that both HslVU and FtsH proteases exhibit an increased affinity for $\sigma^{32}$ at the elevated temperature was not excluded.

Digestion of $\sigma^{32}$ by Chymotrypsin—We then compared the susceptibility of $\sigma^{32}$ to chymotrypsin, known to exhibit a low
Proteolytic activities of HslVU (milligrams of substrate hydrolyzed per h/mg of HslVU under the standard conditions) were determined on the basis of initial reaction rates in experiments similar to those presented in Figs. 3 and 4. Averages of two to four experiments are shown. Values in parentheses indicate activities relative to those at 35 °C with each substrate.

| Substrate | 35 °C | 38 °C | 41 °C | 44 °C |
|-----------|-------|-------|-------|-------|
| σ^{32}    | 0.36 ± 0.05 | 0.67 ± 0.04 | 3.2 ± 0.3 | 5.5 ± 0.3 |
| MBP-SulA  | 4.4 ± 0.1 | 6.2 ± 0.1 | 9.0 ± 0.8 | 10.4 ± 0.5 |
| α-Casein  | 3.1 ± 0.2 | 4.9 ± 0.4 | 7.3 ± 0.5 | 8.9 ± 0.5 |

Fig. 4. Proteolysis of SulA and α-casein by HslVU at various temperatures. MBP-SulA fusion protein (A) and α-casein (B) were used as substrates for the HslVU protease under conditions identical to those employed for σ^{32}, and samples taken at intervals were analyzed as described in the legend to Fig. 3. ○, 35 °C; △, 38 °C; □, 41 °C; ●, 44 °C.

Fig. 5. Degradation of σ^{32} and α-casein by FtsH protease. σ^{32} (4 μg; A) or α-casein (15 μg; B) was incubated with FtsH (1 μg) in the reaction mixture for FtsH (25 μl) at 35 °C (○) or 44 °C (●) (upper panels). Samples (4 μl) withdrawn at intervals were analyzed by SDS-PAGE followed by Coomassie Brilliant Blue staining as described in the legend to Fig. 3. Bands of σ^{32} and α-casein in A and B, respectively, were quantified, and the amount of proteins degraded is presented as percent of the total substrate used (lower panels).

The results obtained in vitro with two proteases that can degrade σ^{32}, we addressed the question of whether the in vivo turnover of σ^{32} reflects the observed high susceptibility of σ^{32} to proteolysis at high temperature. Thus, the half-life of σ^{32} was determined with exponentially growing cells at different temperatures by pulse-chase experiments, followed by immunoprecipitation with specific antisera. When wild-type cells (MG1655) grown at 30, 37, and 42 °C were pulse-labeled with [35S]methionine and chased with excess unlabeled methionine, the half-life of σ^{32} showed a clear dependence on temperature: 1 min at 30 °C, 20 s at 37 °C, and 10–15 s at 42 °C (Fig. 6A), indicating that σ^{32} is extremely unstable at high temperature.

To further analyze the temperature dependence of σ^{32} degradation in vivo, we also examined the stability of σ^{32} by using the σ^{32} overexpression system. IPTG was added to the exponentially growing cells at 30 °C to induce σ^{32} synthesis directed by the trc promoter on the expression plasmid (pKV1278). After 30 min, chloramphenicol was added to stop further protein synthesis, and the culture was divided into two, one kept at 30 °C and the other shifted to 42 °C. The half-life of σ^{32} was determined by following the remaining levels of σ^{32} by immu-
were added at the end of a 2-h incubation. Samples (10 µl) were taken at the indicated (left to right). In all protocols, HslV and HslU results seem to be quite consistent with those obtained de novo assuming that nonnative proteins accumulated upon tempera-
sation. MG1655 cells harboring pKV1278 were grown in synthetic medium at 30, 37, and 42 °C to mid-log phase, pulse-labeled with [35S]methionine (200 µCi/ml) for 30 s, and chased with excess unlabeled methionine (200 µg/ml). Samples were taken as indicated, starting with 0.7 min at 42 °C, 1.3 min at 37 °C, 1.7 min at 30 °C under these conditions. The half-life of σ32 varied with temperature: 0.7 min at 42 °C, 1.3 min at 37 °C, 1.7 min at 30 °C, and 15 min at 22 °C, quite consistent with our results. As shown in Fig. 8D, the overproduced σ32 that was markedly stabilized at 30 °C was rapidly degraded upon a shift to 42 °C in the absence of protein synthesis, indicating that destabilization of σ32 at high temperature does not require de

**FIG. 8. Turnover of σ32 in exponentially growing cells at different temperatures.** A, MG1655 cells were grown in synthetic medium at 30, 37, and 42 °C to mid-log phase, pulse-labeled with [35S]methionine (100 µCi/ml) for 30 s, and chased with excess unlabeled methionine (200 µg/ml). Samples were taken as indicated, starting with the first sample taken at 30 °C (set as time 0), and labeled σ32 was analyzed by immunoprecipitation followed by SDS-PAGE. Analysis of the labeled bands was done with a Fuji BAS2000 imaging analyzer. B, the stability of σ32 overproduced from the expression plasmid was examined. MG1655 cells harboring pKV1278 were grown in synthetic medium at 30 °C to mid-log phase; IPTG (1 mM) was added; and after 30 min, chloramphenicol (100 µg/ml) was added. The culture was divided into two parts; one was kept at 30 °C, whereas the other was shifted to 42 °C (time 0). Samples were taken at 2-min intervals and analyzed for the remaining σ32 levels by immunoblotting.

**FIG. 7. Reversibility of the change in sensitivity of σ32 to HslVU.** σ32 was incubated for 3 h in a reaction mixture (50 µl) according to four different protocols. I and II, σ32 was incubated at constant temperature (control); III and IV, temperatures were changed at 1-h intervals as indicated (left to right). In all protocols, HslV and HslU were added at the end of a 2-h incubation. Samples (10 µl) were taken before (lanes 1, 3, 5, and 7) and after (lanes 2, 4, 6, and 8) addition of the protease and were subjected to SDS-PAGE followed by Coomassie Brilliant Blue staining (upper panel). Bands of σ32 were quantified, and the amount of σ32 remaining is presented as percent of the total σ32 used (lower panel).

The present experiments demonstrated that purified HslVU protease can catalyze the ATP-dependent proteolysis of σ32, as predicted by our previous genetic data (24). As was the case with FtsH (23) and ClpAP (36) proteases, the HslVU protease did not require the presence of DnaK/DnaJ chaperones for activity, although these chaperones are needed for the rapid turnover of σ32 in vivo (15, 16). Since the Lon protease can also degrade σ32 in vitro, most of the known ATP-dependent proteases (possibly except for ClpXP) appear to be capable of hydrolyzing σ32 as their common substrate. Such an overlapping specificity is not unique to σ32 because several other proteins are now known to be degraded by more than one proteases: this includes Xis of phage λ (37), the SsrA-tagged λ repressor (38, 39), and the SulA cell division inhibitor (27, 35).

**DISCUSSION**

The proteolysis of σ32 by HslVU or FtsH showed a marked dependence on high temperature as compared with that of other substrates (Figs. 3–5). The results of chymotrypsin digestion of σ32 suggested a conformational change of σ32 as a basis for the observed differences in susceptibility to the proteases between 35 and 44 °C. Although the exact nature of the change(s) remains obscure, it seems likely that a conformational change occurs at a restricted region of σ32 since σ32 evidently retains much of the activities at 44 °C in vivo (41) and in vitro (Fig. 2) (42). σ32 may exhibit a unique intrinsic instability based on a conformational change at high temperature. Alternatively, the proteases might show increased affinities for σ32 at high temperature, although these possibilities are not mutually exclusive.

In agreement with the results obtained in vitro, the half-life of σ32 in vivo as determined by pulse-chase experiments with normally growing cells turned out to be much shorter at higher temperature than at lower temperature, –1 min at 30 °C and 10–15 s at 42 °C (Fig. 8A). Tilly et al. (15) reported previously, using an overexpression system of σ32, that the half-life of σ32 varied with temperature: 0.7 min at 42 °C, 1.3 min at 37 °C, 1.7 min at 30 °C, and 15 min at 22 °C, quite consistent with our results. As shown in Fig. 8D, the overproduced σ32 that was markedly stabilized at 30 °C was rapidly degraded upon a shift to 42 °C in the absence of protein synthesis, indicating that destabilization of σ32 at high temperature does not require de

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2 H.-C. Huang and A. L. Goldberg, personal communication.
The finding that cells synthesizing the transient stabilization of degradation may be required to counteract the higher rate of temporary adjustment or fine-tuning of its synthesis rate and intrinsic stability, both results combined with other evidence suggest that the intracellular stability of σ^{32} is modulated by its own susceptibility to proteases reflecting ambient temperature, as well as through interaction with the chaperones.

Finally, the present data raise the intriguing question of the potential physiological significance of the unusually high instability of σ^{32} at high temperature. Even when the difference in cellular growth rate at 30 and 42 °C (~2-fold) was taken into consideration, the in vivo turnover of σ^{32} seemed to be appreciably faster at 42 °C. On the other hand, the synthesis rate of σ^{32} at 42 °C appears to be severalfold higher than that at 30 °C (5). The finding that cells synthesizing σ^{32} at a higher rate simultaneously exhibit increased degradation of σ^{32} at high temperature might seem paradoxical. However, the increased synthesis primarily determined by the stability of rpoH mRNA secondary structure (14) would be absolutely needed to rapidly respond to temperature upshift, whereas the higher rate of degradation may be required to counteract the higher rate of synthesis to avoid excessive accumulation of σ^{32}. Although higher levels of σ^{32} are required for cell growth at higher temperature (44), excessive σ^{32} is deleterious and inhibits cell growth (15, 45). We suggest that the basal level of σ^{32} is determined by its synthesis rate and intrinsic stability, both directly reflecting the ambient temperature. In addition, the temporary adjustment or fine-tuning of the σ^{32} level such as the transient stabilization of σ^{32} observed immediately following heat shock is accomplished most effectively by modulating its stability in accordance with the state of protein folding in the cell.

Acknowledgments—We are grateful to W.-F. Wu, S. Gottesman, H.-C. Huang, and A. Goldberg for communicating results prior to publication and to T. Ogura, A. Kiyama, Y. Ishii, and Y. Kato for kind gifts of proteins, antisera, and plasmids. We thank M. Nakayama and S. Takahara for technical assistance.

REFERENCES

1. Yura, T., Nagai, H., and Mori, H. (1993) Annu. Rev. Microbiol. 47, 321–350
2. Gottesman, S., Liberko, K., Zylicz, M., and Ang, D. (1994) in The Biology of Heat Shock Proteins and Molecular Chaperones (Morimoto, R. I., Tissieres, A., and Gottesman, C., eds) pp. 209–249, Cold Spring Harbor Laboratory.
3. Gross, C. A. (1996) in Escherichia coli and Salmonella: Cellular and Molecular Biology (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., and Umbarger H. E., eds) pp. 1382–1399, American Society for Microbiology, Washington, D. C.
4. Yura, T. (1996) Genes Cells 1, 277–284
5. Strauss, D. B., Walter, W. A., and Gross, C. A. (1987) Nature 329, 348–351
6. Goff, S. A., and Goldgerg, A. L. (1985) Cell 41, 587–595
7. Ito, K., Akiyama, Y., Yura, T., and Shiba, K. (1986) J. Bacteriol. 167, 201–204
8. Parsell, D. A., and Sauer, R. T. (1989) Genes Dev. 3, 1226–1232
9. Wild, J., Walter, W. A., Gross, C. A., and Altman, E. (1993) J. Bacteriol. 175, 3992–3997
10. Kanemori, M., Mori, H., and Yura, T. (1994) J. Bacteriol. 176, 5648–5653
11. Nagai, H., Yuzawa, H., and Yura, T. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10515–10519
12. Yuzawa, H., Nagai, H., Mori, H., and Yura, T. (1993) Nucleic Acids Res. 21, 5449–5455
13. Morita, M., Kanemori, M., Yanagi, H., and Yura, T. (1999) J. Bacteriol. 181, 401–410
14. Morita, T., Tanaka, Y., Kodama, T. S., Kyyogoku, Y., Yanagi, H., and Yura, T. (1999) Genes Dev. 13, 655–665
15. Tilly, K., Spence, J., and Georgopoulos, C. (1989) J. Bacteriol. 171, 1565–1589
16. Strauss, D., Walter, W., and Gross, C. A. (1990) Genes Dev. 4, 2002–2009
17. Keller, J. A., and Simon, L. D. (1988) Mol. Microbiol. 2, 31–41
18. Strauss, D. B., Walter, W. A., and Gross, C. A. (1988) Genes Dev. 2, 1851–1858
19. Craig, E. A., and Gross, C. A. (1991) Trends Biochem. Sci. 16, 135–140
20. Bukau, B. (1993) Mol. Microbiol. 9, 671–680
21. Tomoyasu, T., Ogura, T., Tsutsumi, T., and Bukau, B. (1998) Mol. Microbiol. 30, 567–571
22. Herman, C., Thevenet, D., Arai, R., and Bouloc, P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3516–3520
23. Tomoyasu, T., Gainer, J., Bukau, B., Kanemori, M., Mori, H., Rutman, A. J., Oppeheim, A. B., Yura, T., Yamanaka, N., Kishi, H., Hiraga, S., and Ogura, T. (1995) EMBO J. 14, 2551–2560
24. Kanemori, M., Nishihara, K., Yanagi, H., and Yura, T. (1997) J. Bacteriol. 179, 7219–7225
25. Kessel, M., Wu, W.-F., Gottesman, S., Kocsis, E., Steven, A. C., and Maurizi, M. R. (1996) FEBS Lett. 398, 274–278
26. Rohrwill, M., Pfeifer, G., Santarius, U., Muller, S. A., Huang, H.-C., Engel, A., Baumeister, W., and Goldberg, A. L. (1997) Nature Struct. Biol. 4, 133–139
27. Kanemori, M., Yanagi, H., and Yura, T. (1999) J. Bacteriol. 181, 3674–3680
28. Kusukawa, N., and Yura, T. (1988) Genes Dev. 2, 874–882
29. Tohe, T., Ito, K., and Yura, T. (1984) Mol. Gen. Genet. 195, 10–16
30. Liberek, K., Galitski, T. P., Zylicz, M., and Georgopoulos, C. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 5316–5320
31. Gainer, J., Mullhaup, C., Tomoyasu, T., McCarty, J. S., Rudiger, S., Schonfeld, H.-J., Schirra, C., Bujard, H., and Bukau, B. (1996) EMBO J. 15, 607–617
32. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
33. Maurizi, M. R., Thompson, M. W., Singh, S. K., and Kim, S.-H. (1994) Methods Enzymol. 244, 314–331
34. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
35. Wu, W.-F., Zhou, Y.-N., and Gottesman, S. (1999) J. Bacteriol. 181, 3681–3687
36. Wawrysnow, A., Wojtkowiak, D., Marszalek, J., Bancki, B., Jonsen, M., Gravers, B., Georgopoulos, C., and Zylicz, M. (1995) EMBO J. 14, 1867–1877
37. Leffers, G. G., Jr., and Gottesman, S. (1998) J. Bacteriol. 180, 1573–1577
38. Gottesman, S., Roche, E., Zhou, Y.-N., and Sauer, R. T. (1998) Genes Dev. 12, 1338–1347
39. Herman, C., Thevenet, D., Bouloc, P., Walker, G. C., and D’Ari, R. (1998) Genes Dev. 12, 1348–1355
40. Gottesman, S. (1996) Annu. Rev. Genet. 30, 465–506
41. Herendeen, S. L., VanBogelen, R. A., and Neidhardt, F. C. (1979) J. Bacteriol. 139, 185–194
42. Blaszczak, A., Zylicz, M., Georgopoulos, C., and Liberek, K. (1995) EMBO J. 14, 5085–5093
43. Blaszczak, A., Georgopoulos, C., and Liberek, K. (1999) Mol. Microbiol. 31, 157–166
44. Yamamori, T., and Yura, T. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 860–864
45. Bahl, H., Ecchs, H., Strauss, D. B., Court, D., Crowl, R., and Georgopoulos, C. P. (1987) Genes Dev. 1, 57–64

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3 M. T. Morita and T. Yura, unpublished observation.