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Convergence of Molecular, Modeling, and Systems Approaches for an Understanding of the *Escherichia coli* Heat Shock Response

Eric Guisbert,[1]† Takashi Yura,[2] Virgil A. Rhodius,[2] and Carol A. Gross[2,3]*

Departments of Biochemistry and Biophysics,[1] Microbiology and Immunology,[2] and Cell and Tissue Biology,[3] University of California at San Francisco, San Francisco, California 94158

INTRODUCTION

The heat shock response (HSR) is classically defined as the cellular response to temperature increase. A major component of this response is the upregulation of a set of proteins termed heat shock proteins (hsp's). These proteins are usually regulated by a single transcription factor, for example, the σ^{32} transcription factor in *Escherichia coli* and heat shock factor (HSF) in eukaryotic cells. Some hsp's, notably chaperones, which help proteins fold, and proteases, which degrade unfolded proteins, are a conserved part of the response from bacteria to humans. The rapid upregulation of chaperones and proteases during the HSR restores an appropriate protein-folding environment in the cell, suggesting that maintaining protein-folding homeostasis is a primary function of the σ^{32}/HSF-mediated HSR. Consistent with this idea, many other treatments that destabilize folded proteins or make it more difficult for nascent proteins to fold also activate this response.

In this review, we discuss the regulation and function of the *E. coli* σ^{32}-mediated HSR. We show that this response is subject to complex regulation, which is currently not completely understood. In addition, we discuss the broad reach of the σ^{32} regulon, which encodes functions that alter multiple cellular processes to permit increased survival in response to high temperature.

A PHYSIOLOGICAL DESCRIPTION OF THE HSR

The σ^{32}-mediated HSR has been characterized by examining the behavior of the response after both upshift and downshift in temperature. Following upshift to temperatures of ≥37°C but within the growth range of the organism, the synthesis of hsp's first increases rapidly during the induction phase of the response, then declines during the adaptation phase of the response, and finally achieves a new steady-state level characteristic of the particular high temperature (Fig. 1A) (53). The initial rapid increase in synthesis allows hsp's to reach the level characteristic of the new temperature rapidly. Conversely, following temperature downshift, hsp synthesis abruptly decreases about 20-fold and then gradually increases over the next several doublings until it reaches the rate characteristic of 30°C (Fig. 1B) (52). Excess hsp's are diluted out as cells grow; it is believed that the normal rate of hsp synthesis resumes when the cellular level of hsp's approximates that characteristic of growth at 30°C.

Three regulatory loops control the output by altering the level and activity of σ^{32} (Fig. 2) (reviewed in reference 67). First, translation of σ^{32} increases at high temperature. Second, σ^{32} stability is controlled: σ^{32} is rapidly degraded during steady-state growth at both low and high temperatures but transiently stabilized after shift to high temperature. Finally, σ^{32} activity is negatively regulated. Activity control is believed to adjust σ^{32}-mediated transcription to a rate appropriate for the level of unfolded proteins present in the cell, leading to the transient inactivation of σ^{32} when excess hsp's are present. During the induction phase of the HSR, increased translation and transient stabilization of σ^{32} result in a rapid increase in its level (Fig. 1A), while the high pool of unfolded proteins removes the negative regulation of σ^{32} activity. Together, these mechanisms account for the rapid increase in hsp synthesis during the induction phase. During the adaptation phase, activity control is believed to mediate the decline in hsp synthesis that precedes the decline in σ^{32} levels (Fig. 1A). Likewise, following temperature downshift, activity control is believed to mediate the decline in hsp synthesis that precedes the decline in σ^{32} levels (Fig. 1B). The effect of each regulatory loop on response performance has been modeled; these data will be discussed later in the review.

* Corresponding author. Mailing address: Genentech Hall, Room S372E, 600 16th Street, University of California at San Francisco, San Francisco, CA 94158-2517. Phone: (415) 476-4161. Fax: (415) 514-4080. E-mail: cgross@cgl.ucsf.edu.

† Present address: Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL 60208.

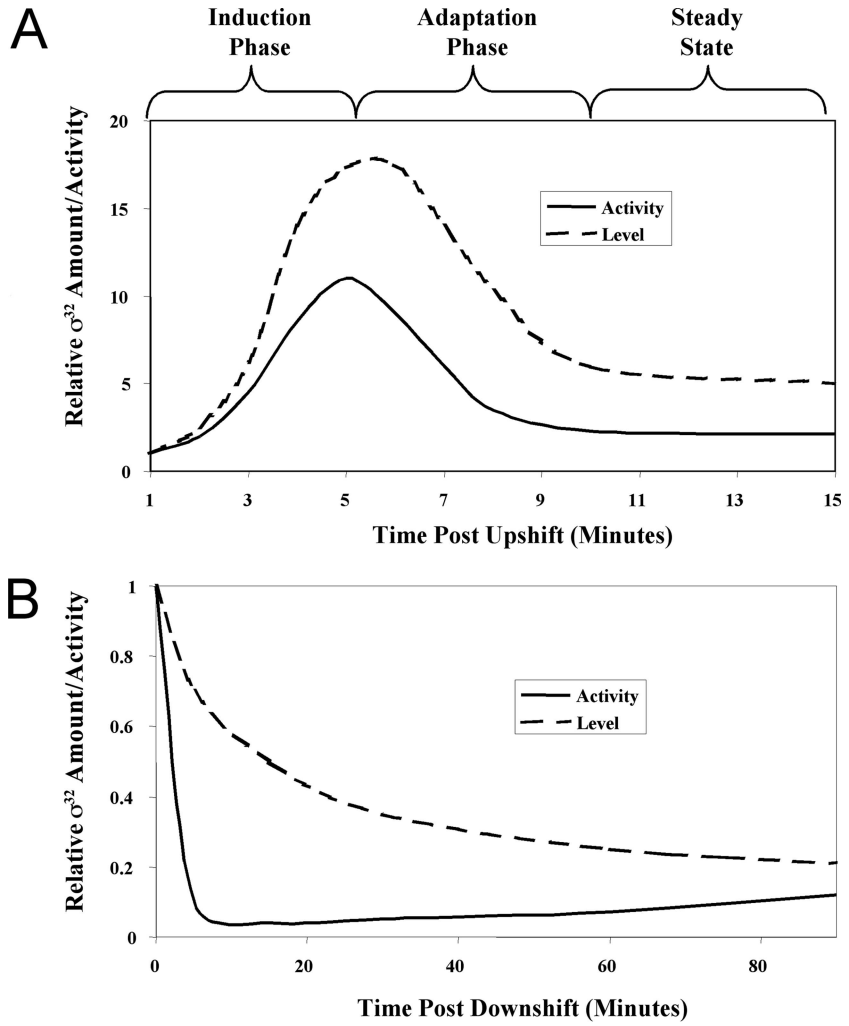

FIG. 1. Activation and repression of the HSR during temperature upshift and downshift. (A) Activation of the HSR during a temperature shift from 30 to 42°C reveals three distinct phases: induction, adaptation, and steady state. (B) Repression of the HSR during a temperature shift from 42° to 30°C. The relative σ^{32} activities measured by HSP synthesis are shown by the solid lines; relative σ^{32} levels measured by Western blotting analysis are shown by the dotted lines.

TRANSLATIONAL CONTROL OF σ^{32}

Early studies demonstrated that the rate of translation of σ^{32} was controlled by temperature: σ^{32} synthesis was 5- to 10-fold higher at 42°C than at 30°C (14, 39, 40, 53). A structural transition in *rpoH* mRNA encoding σ^{32} mediates this control: at low temperature, base pairing within *rpoH* mRNA occludes the Shine-Dalgarno sequence and the translation start point of σ^{32}, resulting in inefficient initiation of translation; at high temperature, this inhibitory structure is melted so that the translation of σ^{32} increases (35, 37). The evidence for this view is as follows: (i) destabilizing the predicted inhibitory structure by single base changes increases the translation of σ^{32} at low temperature, whereas stabilizing the inhibitory structure with single base changes decreases the translation of σ^{32} at all temperatures; and (ii) temperature regulation and the effects of stabilizing and destabilizing mutations can be reproduced in vitro by assaying ribosome binding to *rpoH* mRNA. These experiments suggest that translation control responds directly to changes in temperature rather than sensing the cellular folding environments that result from the temperature changes. It is not known whether additional factors or stresses alter the performance of this switch in vivo.

REGULATION OF σ^{32} ACTIVITY

Control of σ^{32} activity was initially inferred from the fact that under conditions where there is transiently more σ^{32} than necessary, expression of hsp's is lower than expected from the amount of σ^{32} present in the cell (52). Thus, activity regulation is observed during the adaptation phase of temperature upshift (Fig. 1A), after temperature downshift (Fig. 1B), and when σ^{32} is artificially overexpressed. The "unfolded protein titration model" has been proposed to explain activity control. According to this model, σ^{32} samples chaperone activity to sense the amount of unfolded proteins in the cell: when chaperone levels are abundant relative to unfolded proteins, the chaperones would feedback inhibit σ^{32} and therefore negatively regulate further chaperone production. Consistent with this idea, the

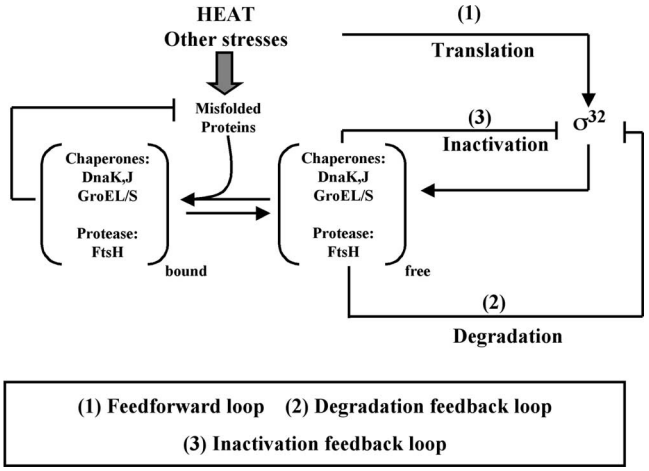

FIG. 2. Wiring diagram of σ^{32} regulation. There are three primary modes of regulation as follows: (i) excess free DnaK/J and GroEL/S chaperones directly bind to and inactivate σ^{32}; (ii) the FtsH protease degrades σ^{32}, with chaperones participating in this process; and (iii) temperature directly controls the rate of σ^{32} translation. Misfolded proteins titrate chaperones from these regulatory roles, allowing active σ^{32} to increase the synthesis of chaperones and proteases during conditions where they are needed.

overexpression of either the DnaK/J or the GroEL/S chaperone machine inactivates σ^{32}; conversely, depleting either chaperone machine leads to the accumulation of active σ^{32} (16, 58). The DnaK/J chaperone machine, consisting of the Hsp70 homologue DnaK and its Hsp40 cochaperone DnaJ, and the GroEL/S chaperone machine, consisting of the Hsp60 homologue GroEL and its Hsp10 cochaperone GroES, represent the two major cytoplasmic chaperones in *Escherichia coli*. The strongest evidence supporting the "unfolded protein titration model" is that the expression of unstable proteins that titrate either DnaK/J or GroEL/S induces the HSR (16, 58). This suggests that σ^{32} is not responding to the total level of chaperones in the cell but rather is responding to the ratio of chaperone relative to those of its unfolded protein substrates. This control circuit would allow the cell to continuously monitor its protein-folding state to ensure that the expression of chaperones was appropriate for the unfolded substrate load. Consistent with this model, chaperones do not participate in stable interactions with their substrates; rather, they engage in cycles of substrate binding and release that are driven by the ATPase activity of the chaperones.

The molecular mechanism of the chaperone-mediated inactivation of σ^{32} is not completely settled. The prevalent model is that the chaperones effectively act as anti-sigma factors and simply compete with RNA polymerase for binding to σ^{32}. In support of this idea, both DnaK/J and GroEL/S bind to σ^{32} in vitro, and the addition of either DnaK/J or GroEL/S to an in vitro transcription reaction mixture containing purified σ^{32} and RNA polymerase leads to a decrease in σ^{32}-dependent transcription (10, 11, 16, 32). However, there are accumulating hints that the inactivation mechanism may be more complex than simple competition. First, σ^{32} binds to GroEL/S with an affinity ~1,000× lower than that with which it binds to RNA polymerase. Yet, a small excess of GroEL/S over RNA polymerase (~5-fold) efficiently inactivates σ^{32} in vitro, which cannot be explained by a competitive binding model (16). Second, although in vitro binding studies indicate that GroEL/S binds ~100-fold more weakly to σ^{32} than does DnaK/J, GroEL/S is at least as efficient as DnaK/J in mediating inactivation both in vivo and in vitro (16). Finally, point mutants have been identified in σ^{32} that are resistant to inactivation by both GroEL/S and DnaK/J in vivo, but these mutants are inactivated indistinguishably from the wild type in the current in vitro inactivation assay (these mutants are discussed further in the section on σ^{32} structure/function). Together, these observations raise the possibility that an additional component(s) may be involved in inactivation.

Thus far, we have considered only the action of the major Hsp40 family member, DnaJ, in mediating activity control. However, *E. coli* has other Hsp40s, and one of them, CpbA, is known to be able to mediate activity control in vivo in collaboration with DnaK (60, 61). It is not known to what extent σ^{32} is regulated by DnaK/J versus DnaK/CbpA. Moreover, CbpA has not been examined in vitro in regard to σ^{32} binding or inactivation. Also, CbpA activity can be modulated in vivo by the accessory factor CbpM (5). It will be interesting to determine the conditions under which CbpA and CbpM are important in mediating activity control.

There is another potential contribution to the activity control mechanism just described. The DnaK/J chaperone machine requires the GrpE nucleotide exchange factor to exchange ADP for ATP, therefore allowing substrate release and a new round of substrate binding. There is evidence that GrpE can act as a thermosensor (reviewed in reference 65). At high temperatures, GrpE activity decreases, leading to a slower ATPase cycle, which in turn leads to DnaK/J acting more like holdase than a foldase. Whether this alteration in the functional properties of GrpE affects the activity control of σ^{32} has not been investigated.

Finally, the cell can adapt to long-term imbalances in the ratio of chaperones to unfolded proteins. Whereas the overexpression of either DnaK/J or GroEL/S results in an immediate inhibition in σ^{32} activity, following long-term (20-h) overexpression of chaperones, the σ^{32} activity level is upregulated to approximately that of wild-type cells (17). Adaptation is accomplished without the downregulation of the overexpressed chaperones. The players in this regulatory loop are currently unknown.

DEGRADATION CONTROL OF σ^{32}

The degradation control of σ^{32} is complex. σ^{32} is degraded rapidly during steady-state growth, exhibiting a half-life of ~1 min at low temperature (30°C) and an even higher rate of degradation (half-life of ~20 s) at high temperature (42°C) (27, 36). In addition, immediately after a shift to high temperature, this normally unstable protein is transiently stabilized for a 5- to 10-min period (53). Currently, some but not all features of this response are understood.

A search for the factors involved in the degradation of σ^{32} indicated that FtsH (HflB), an ATP-dependent protease localized to the inner membrane, is the major protease that degrades σ^{32}. σ^{32} is almost completely stable in cells lacking FtsH, whereas single deletions of other cytoplasmic ATP-dependent proteases have little or no effect on σ^{32} stability (20,

26, 57). In addition, the DnaK/J and GroEL/S chaperone machines are implicated in σ^{32} degradation, as depleting either chaperone machine or mutationally inactivating DnaK or DnaJ stabilizes σ^{32} (16, 51, 58). The involvement of chaperones in σ^{32} degradation means that the "unfolded protein titration model" could explain the transient stabilization of σ^{32} after temperature upshift as well as the activity control of σ^{32}. The increased prevalence of unfolded proteins after a shift to high temperature would titrate chaperones away from their role in degradation so that σ^{32} would be transiently stabilized. If so, this would allow the rate of degradation to directly monitor the folding status of the cell. Additionally, the increased prevalence of FtsH substrates after temperature upshift could titrate FtsH from degrading σ^{32}, resulting in its transient stabilization, thereby tying the degradation rate of σ^{32} to substrate flux through FtsH. However there are conflicting data about whether FtsH is a limiting component in the degradation reaction (20, 55).

Several groups have investigated the thermal behavior of σ^{32}. Both protease sensitivity and hydrogen-deuterium exchange experiments coupled with mass spectrometry indicate that σ^{32} becomes more unstructured at high temperatures (47). This may partially explain the extremely rapid degradation of σ^{32} at high temperatures. In addition, FtsH itself has increased activity at higher temperatures (19, 27).

Importantly, the degradation system has not been completely reconstituted in vitro. σ^{32} is degraded very rapidly in vivo, but degradation by FtsH in vitro is very slow (20, 57). Moreover, the DnaK and DnaJ chaperones do not facilitate the degradation of σ^{32} in vitro (3). Investigation of the properties of FtsH revealed that it has a very poor unfoldase activity both in vivo and in vitro, so that it essentially waits for proteins to spontaneously unfold before degrading them (19). The low rate of σ^{32} degradation in vitro could reflect the time required for the unfolding of σ^{32}. Intriguingly, FtsH is a member of the AAA family of proteins, many of which utilize adaptor proteins to modulate their activity (reviewed in reference 7). For example, the degradation of σ^S by the AAA^+ protease ClpXP requires the RssB adaptor protein (69). The putative FtsH adaptor protein(s) missing from the in vitro system could provide unfoldase activity and/or recruit chaperones.

The role of chaperones in σ^{32} degradation is not settled. One prevalent model is that chaperones facilitate σ^{32} degradation by competing with RNA polymerase for binding to σ^{32}. In support of this model, RNA polymerase prevents the degradation of σ^{32} by FtsH in vitro, and the addition of chaperones reverses this effect (3). However, it has recently been shown that chaperones are still required for the in vivo degradation of σ^{32} mutants that are defective in RNA polymerase binding (54).

σ^{32} STRUCTURE/FUNCTION

σ^{32} is a member of the bacterium-specific σ transcription factor family (reviewed in reference 15). All σ's contain binding determinants both for RNA polymerase and for promoter DNA. Binding of a σ to RNA polymerase induces changes both in the σ and in RNA polymerase; the resultant holoenzyme is competent to bind to promoters specified by the particular σ factor utilized. Bacteria generally contain a single

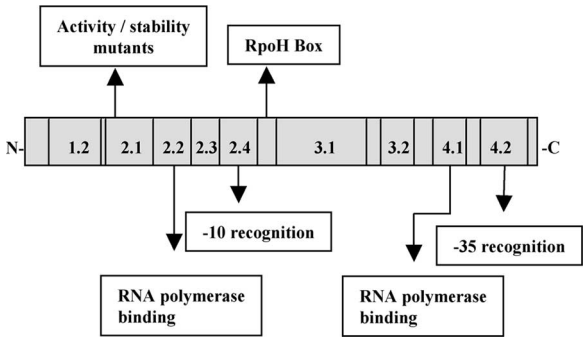

FIG. 3. The domain structure of σ^{32}. A schematic map reveals the domain structure and conserved regions of σ^{32}. Note that domains are divided into subdomains as follows: domain 2 is comprised of subdomains 1.2 to 2.4 and encompasses amino acids 16 to 126, domain 3 is comprised of subdomains 3.1 and 3.2 and encompasses amino acids 127 to 177, and domain 4 is comprised of subdomains 4.1 and 4.2 and encompasses amino acids 213 to 280. The RpoH box is comprised of amino acids 132 to 141. Regions that bind RNA polymerase and promoter DNA are shown below the schematic; features specific to σ^{32} are indicated above the schematic. "Activity/stability mutants" marks the position of mutations in σ^{32} that affect the stability and/or activity of σ^{32}.

housekeeping σ factor and several alternative σ's which mediate responses to altered environmental conditions. σ's contain between two to four domains, depending on the particular group to which they belong. Housekeeping σ's are the most complex and contain four domains; σ^{32}, a member of the group 3 σ's, contains domains 2,3, and 4 (Fig. 3). Several domains contain recognition determinants for RNA polymerase binding and for promoter recognition. Domain 2 recognizes the -10 region of the promoter and carries the major RNA polymerase recognition determinants, whereas domain 4 recognizes the -35 region of the promoter. There is a reasonable amount of knowledge about the RNA polymerase and promoter recognition determinants in σ^{32} both as a result of direct studies on σ^{32} and by extrapolation from studies on other σ's.

In addition to carrying out the functions common to all σ's, σ^{32} must contain determinants that allow it to bind to several chaperones and to the FtsH protease, so that its activity and stability can be properly regulated. However, it has proven surprisingly difficult to identify mutations that are specifically altered in its regulatory determinants. One reason for this is that the multiple regulatory loops tend to obscure the true phenotype of such mutations. For example, mutations in σ^{32} that eliminate the binding site for the FtsH protease would result in the accumulation of high levels of σ^{32} but would not significantly increase σ^{32} activity, because activity control would inactivate excess σ^{32}.

The initial search for a region of σ^{32} specialized to carry out regulatory functions focused on the RpoH box (part of the previously described region C) (38, 41). As this region is unique to σ^{32} and its orthologues in other bacteria, it was an excellent candidate for a region devoted to functions unique to σ^{32}. The RpoH box spans amino acids 122 to 144 and is located at the N terminus of domain 3 (Fig. 3). Two peptides from within the RpoH box region bind DnaK, but it is unlikely that these are used as DnaK binding determinants in intact σ^{32} because mutating these sites did not lead to defects in σ^{32}

TABLE 1. Mutations that affect the stability and/or activity of σ^{32}

Mutation	Alteration(s)	Reference(s) with identification
L47A	Stability	21
L47Q	Stability	21 and 45
L47Q-L55Q	Activity, stability	21
A50D	Activity, stability	66
A50S	Stability	21
A50T	Stability	21
A50V	Stability	45
K51E	Activity, stability	21 and 66
I54A	Activity, stability	21
I54F	Stability	45
I54N	Activity, stability	66
I54T	Activity, stability	21, 45, and 66
L55Q	Stability	21
R91H	Activity, stability	66
R91P	Activity, stability	66
Δ49-52	Activity, stability	66

regulation (1, 33). A frameshift mutation spanning the RpoH box stabilized σ^{32} (38); later studies showed that this peptide is a substrate for FtsH (1), making this a candidate for the FtsH recognition sequence. However, analysis of the various *rpoH* paralogues from *Bradyrhizobium japonicum*, which contains both stable and unstable σ^{32}s, indicated that differences in degradation control did not map to the RpoH box (62). Thus, there is no strong evidence that the RpoH box is involved in either chaperone or protease binding to σ^{32}. At present, the only known function of the RpoH box is binding to RNA polymerase (1, 25).

A great deal of attention has been focused on mapping the degradation determinants in σ^{32}. Motivated by the finding that ATP-dependent proteases often use C-terminal recognition determinants, the role of the C terminus of σ^{32} in degradation control was investigated. Initial studies showed that C-terminal truncations of 15 or 20 amino acids led to the stabilization of σ^{32} both in vivo and in vitro but did not affect DnaK/J binding (3). However, these truncations had additional vector-encoded amino acids added to their C termini. When C-terminal truncations of 5, 11, 15, or 21 amino acids without additional vector sequences were analyzed, these proteins exhibited the same high rate of degradation in vivo as wild-type σ^{32} (56). Moreover, FtsH does not efficiently degrade a peptide derived from the C terminus of σ^{32}. Another approach to mapping the degradation control region utilized chimeras between *E. coli* σ^{32} and its *B. japonicum* orthologue, *rpoH1* (2). The σ^{32} encoded by *rpoH1* is 10 times more stable than *E. coli* σ^{32}, although the two proteins are 40% identical. This work suggests that the main degradation tag lies somewhere between amino acids 36 and 134.

Several groups have used forward genetic screens to search for σ^{32} mutants with alterations in either activity or stability (21, 45, 66). Importantly, these diverse screens with different endpoints have converged on a small region within domain 2.1 of σ^{32}. In one screen, the degradation control phenotype was completely uncoupled from activity control by assaying for the expression of a chimeric adenylyl cyclase whose N and C termini were separated by σ^{32} (45). Because σ^{32} is unstable, the chimeric protein is degraded and adenylyl cyclase activity is low; mutations in σ^{32} resulting in a degradation defect will have higher adenylyl cyclase activity. Importantly, screens for σ^{32} mutants defective in activity regulation found similar affected residues (21, 66). Residues identified in all of the forward genetic screens are shown in Table 1. Analysis of the mutant most defective in activity control (σ^{32} I54N) indicated that it was almost unaffected by the overexpression of either DnaK/J or GroEL/S and simultaneously completely defective in degradation control (66). Activity and degradation control are linked because both processes require chaperone binding. Yet, this mutant showed essentially normal binding to DnaK, DnaJ, and GroEL and was also almost normal in its binding to RNA polymerase. This regulatory region may affect a step downstream of chaperone binding that is important for both processes. For example, it may orchestrate a conformational change or bind an unknown factor. Although the precise defect of these mutants is unknown, the analysis performed thus far indicates that these mutants identify a critical regulatory region within σ^{32} that is important for both activity and stability control.

MODELING REGULATION OF THE HSR

The σ^{32}-mediated HSR is an attractive candidate for mathematical modeling because it is well studied experimentally and known to be subject to complex regulation. Several groups have modeled the response (8, 9, 31, 49). Perhaps the most interesting results were obtained by considering the HSR in the context of control engineering, a discipline that uses modular decomposition to make systems tractable for analysis. In this representation, temperature-regulated translation is considered to be a feed-forward module allowing the system to respond to change in temperature before cellular processes are altered (Fig. 4). Additionally, two feedback loops, one medi-

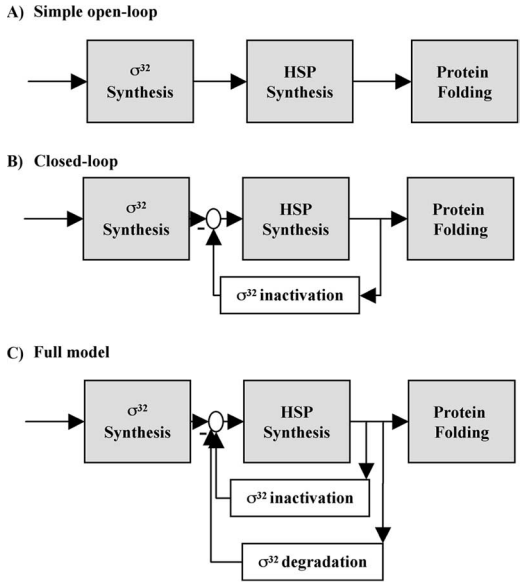

FIG. 4. Modeling the HSR. (A) A simple open-loop design containing only a feed-forward element that senses temperature. (B) A closed-loop design with feed-forward and inactivation loops. (C) A full model containing feed-forward, inactivation, and degradation loops. (Reprinted from reference 9 with permission of the publisher. Copyright 2005 National Academy of Sciences, U.S.A.)

TABLE 2. Localization and functional classification of σ^{32} regulon products[a]

Location or functional category (no. of products)	Regulon products
Location	
Periplasm (8)	CreA[c], DsbC[c], YceI[c], YciM[b], YehR[e], YehZ[e], YgcI[e], YibG[e]
Inner membrane (27)	CreC[c], CutE[c], CycA[b], FtsH[d], FxsA[b,c,e], GntY[c], HflX[d], HflK[d], HflC[d], HtpX[d], LipB[c], LspA[c], MacB[b], MenA[d], PgpA[c], PhoQ[c], SdaC[e], YafU[e], YbeX[c], YbeZ[b,c,e], YceJ[c], YcjF[b,c], YciS[b,c,e], YdgR[e], YghJ[e], YiaA[e], YtfL[e]
Cytoplasm (88)	ClpB[d], ClpP[d], ClpX[d], CreB[c], Crr[e], DnaJ[d], DnaK[d], FkpB[c], FimB[e], FolP[d], GapA[d], GlnS[c,e], GntX[c,e], GroELS[d], GrpE[d], HepA[c,e], Hfq[c], HolC[c,e], Hsp33[c], HslU[d], HslV[d], HtpG[d], IbpA[d], IbpB[d], IleS[c,e], IspH[c], LdhA[b,e], Lon[d], MiaA[d], Mfd[b,e], Mlc[d], MutL[d], MutM[b,c], NarP[c,e], NrdH[e], NusB[c], PhoP[c,e], PrlC[b,c,e], PyrF[c], RecA[c], RecJ[c], RdgB[c], RibE[c], RluA[b], RpmE[e], RpoD[d], RpsL[e], RrmJ[d], *rrnB*[d], SdaA[c,e], ThiL[c], TopA[d], TreR[e], TyrR[c], ValS[c], XerD[c], YadF[c,e], YafD[c], YafE[b,c], YbbN[b,e], YbeD[b,c,e], YbeY[c], YbjX[e], YccE[e], YccV[b,e], YceP[b,c,e], YciH[c], YcjX[b,c,e], YdaM[b], YdeO[e], YdhQ[b,c,e], YeaD[d], YfjN[c], YfiA[e], YgaD[c], YgbF[c], YggW[c], YhdN[c,e], YhiQ[b,c], YibA[b,e], YjhI[e], YjiT[e], YnfK[d], YrdA[b,e], YrfH[b,c], YrfG[c,e], XapR[e], ZntR[c]
Unknown (3)	yi81_1[d], yi82_1[d], YpjM[e]
Functional category	
Metabolism (22)	CutE[c], FolP[d], GapA[d], IspH[c], LdhA[b,e], LipB[c], LspA[c], MenA[d], NrdH[e], PgpA[c], PyrF[c], RibE[c], SdaA[c,e], ThiL[c], YadF[c], YafE[b,c], YceJ[c], YdaM[b], YggW[c], YibA[b,e], YrdA[b,e], YrfG[c,e]
Chaperone/folding catalysts (12)	ClpB[d], DnaJ[d], DnaK[d], DsbC[c], FkpB[c], GroELS[d], GrpE[d], Hsp33[c], HtpG[d], IbpA[d], IbpB[d], YbbN[b,e]
Protein degradation (11)	ClpP[d], ClpX[d], FtsH[d], HflX[d], HflK[d], HflC[d], HslU[d], HslV[d], HtpX[d], Lon[d], PrlC[b,c,e]
DNA modification (9)	HolC[c,e], Mfd[b,e], MutL[d], MutM[b,c], RecA[c], RecJ[c], RdgB[c], TopA[d], XerD[c]
RNA state (3)	Hfq[d], RluA[b], YfjN[c]
Transcription regulators (18)	CreB[c], CreC[c], CycA[b], FimB[e], HepA[c,e], MacB[b], Mlc[d], NarP[c,e], NusB[c], PhoP[c,e], PhoQ[c], RpoD[d], TreR[e], TyrR[c], XapR[e], YdeO[e], YjhI[e], ZntR[c]
Translation/tRNA (11)	GlnS[c,e], IleS[c,e], MiaA[d], RpmE[e], RpsL[e], RrmJ[d], *rrnB*[c], ValS[c], YciH[c], YfiA[e], YrfH[b,c]
Transporter (7)	Crr[c], GntX[c,e], SdaC[e], YbeX[c], YbeZ[b,c,e], YdgR[e], YehZ[e]
Miscellaneous (6)	FxsA[b,c,e], GntY[c], YccV[b,e], YhiQ[b,c], yi81_1[d], yi82_1[d]
Unknown function (27)	CreA[c], YafD[c,e], YafU[e], YbeD[b,c,e], YbeY[c], YbjX[e], YccE[e], YceI[c], YceP[b,c,e], YciM[b,c], YciS[b,c,e], YcjF[b,c], YcjX[b,c,e], YdhQ[b,c,e], YeaD[d], YehR[e], YgaD[c], YgbF[c], YgcI[e], YghJ[e], YhdN[c,e], YiaA[e], YibG[e], YjiT[e], YnfK[d], YpjM[e], YtlL[e]

[a] The predicted functions (http://ecocyc.org) and locations (46) were obtained for all identified σ^{32} regulon products. Note that *rrnB* is an RNA. Underlined proteins are annotated as "membrane"; however, it is assumed that they are located in the inner membrane.
[b] Identified in reference 67.
[c] Identified in reference 43. Only regulon products encoded on the chromosome and with a confirmed σ^{32}-dependent promoter are listed.
[d] Previously known; referred to in reference 43.
[e] Identified in reference 62.

ating activity control and a second mediating degradation control, report on cellular conditions, allowing a homeostatic response. To examine the function of each of these modules, the response properties of "virtual mutants" that had various combinations of these modules were modeled. This analysis revealed that regulation is not redundant; instead, each module contributes different features to the response. The simplest system is one in which control is exerted solely by a direct sensor of temperature (temperature-regulated translation); as σ^{32} increases, chaperones and proteases increase concomitantly. This system can achieve any output desired but does not respond to the internal state of the cell because it contains no feedback loops. Such a system is inefficient because it utilizes many chaperones to accomplish folding at elevated temperatures even if the level of unfolded proteins is low, and it is also very sensitive to parameter variation—the system changes in concert with changes in parameters. The addition of activity control improves the efficiency of the system and makes it less sensitive to parameter variation. The further addition of degradation control improves the kinetics of the response, increases its efficiency, and reduces cell-to cell variation. Analyses of this type rationalize the complexity of biological control mechanisms. The availability of such a detailed model begs for experimental tests of these predictions. Should the experimental tests fail to validate the model, this would suggest that additional control features are present, thereby motivating a new round of investigation of the response. Likewise, the identification of additional control mechanisms experimentally would motivate the development of a new model.

THE FUNCTIONS OF THE σ^{32} REGULON

The σ^{32}-mediated HSR is the most immediate response of *E. coli* and related organisms to temperature stress. Therefore, identifying the functions encoded by this regulon will allow us to determine the cellular alterations that permit adaptation to this ubiquitous stress. Recently, the functions encoded by the regulon have been identified either by using whole-genome expression analysis to identify RNAs that increase after overexpression of σ^{32} and transfer to high temperature (44, 68) or by examining σ^{32} holoenzyme binding to DNA after temperature upshift by use of chromatin immunoprecipitation coupled with microarray analysis (63). The cellular location and functions of proteins known or likely to be part of the σ^{32} regulon are presented in Table 2.

The earliest identified members of the regulon were chaperones, suggesting that maintaining protein homeostasis was an important role of the regulon (reviewed in references 12 and 13). The centrality of this function of the HSR is evidenced by the fact that these functions are under heat shock control in organisms as diverse as bacteria and humans. However, analysis of regulon members reveals a new dimension in protein

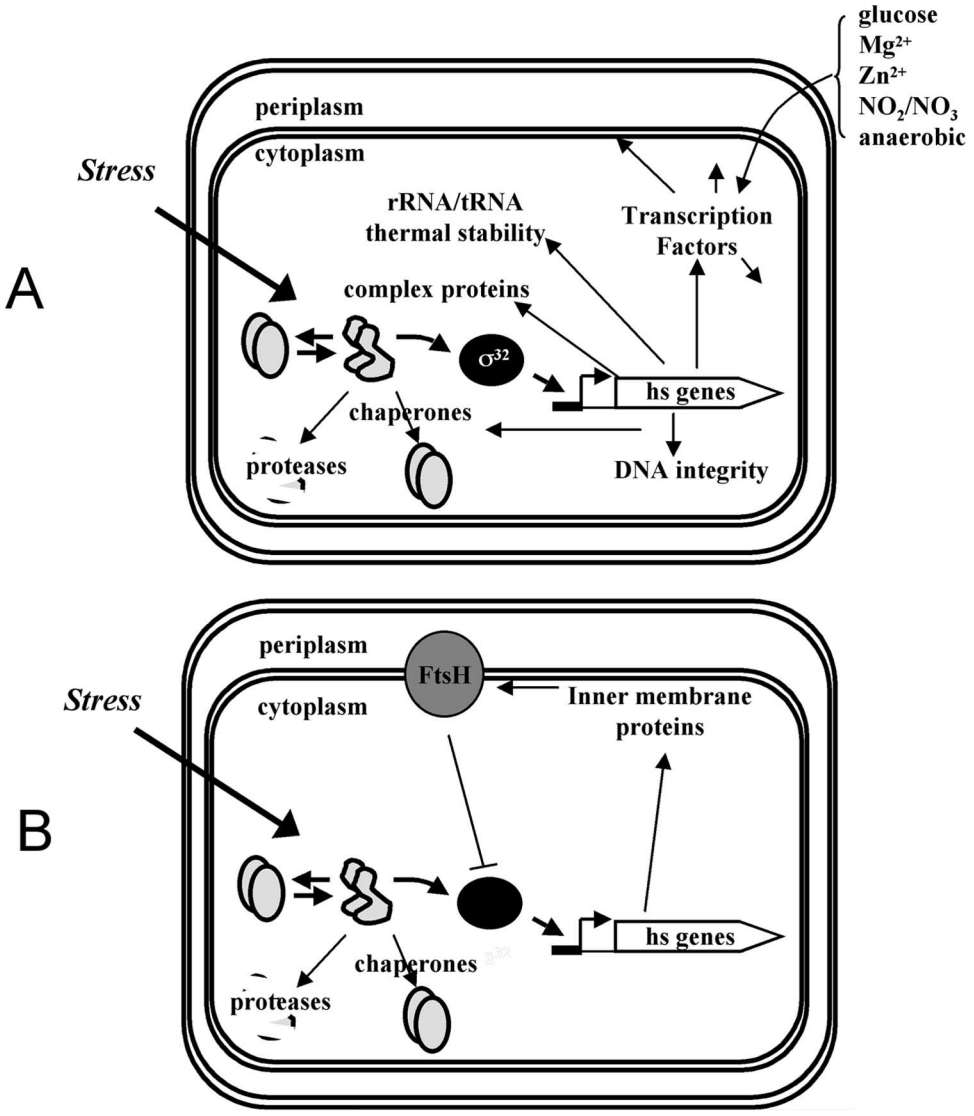

FIG. 5. Functions of the HSR. The induction of σ^{32} and protein products of the target heat shock (hs) genes are shown in a model of the *E. coli* cell, illustrating the compartmentalization of the response. (A) The σ^{32} regulon protects many cytoplasmic molecules and processes, including transcription factors. The environmental cues that regulate the transcription factors are indicated next to the curved brace. (B) The σ^{32} regulon also protects cytoplasmic membranes and inner membrane proteins. Note that the overexpression of many inner membrane proteins also induces the σ^{32} response.

homeostasis, complex cellular proteins containing moieties including iron-sulfur clusters, lipoyl modifications, and cofactors. It is perhaps not surprising that many members of the regulon are devoted to the homeostasis of complex proteins, as these proteins may be particularly at risk after a switch to high temperature, and regeneration pathways for some of these proteins have not been identified. The regulon encodes at least two iron-sulfur cluster proteins; it is possible that the synthesis of these proteins compensates for their destruction (reviewed in reference 23). Additionally, the regulon encodes proteins involved in lipoyl biosynthesis, iron-sulfur assembly (GntY, a homologue of IscA), and cofactor biosynthesis, thus ensuring a sufficient flux of the building blocks for complex proteins.

In addition to protecting proteins from destruction, functions encoded by the σ^{32} regulon also protect other macromolecules and cellular processes (Fig. 5A). A number of regulon functions protect cellular DNA; the regulon includes Mfd, which recruits repair machinery to DNA lesions, as well as enzymes involved in three different pathways that maintain genomic integrity (mismatch repair, excision repair, and recombination) (48). Enzymes for modifying rRNA (23S methylation) and tRNA (Δ^3-isopentyl diphosphate added adjacent to the anticodon) are encoded by the regulon; these modifications are believed to be important for growth at high temperature (4, 59). Several RNA polymerase binding proteins (HepA, TopA, and NusB) believed to be important for alleviating the effects of supercoiling on transcription and two proteins implicated in ribosome protection and recycling (YfrH [Hsp15] and YfiA) are regulon members (6, 29, 30, 50). Taken together, this brief survey indicates that σ^{32} regulon members

protect DNA and RNA in addition to proteins from the deleterious effects of excess heat and also modify the transcription, translation, and repair machinery to enable stress adaptation.

Global analysis of the regulon indicated that it encodes many transcription factors in addition to the RNA polymerase binding proteins that we have just enumerated. In fact, about 12.5% of the regulon is devoted to transcription factors, and the percentage goes up to 15% if one considers Mfd and TopA, which bind to RNA polymerase and modify its transcription properties, and FimB, which inverts a DNA segment to modify the rate of switching to the on or off transcriptional state for fimbrial synthesis. This proportion is slightly higher than the fraction of the σ^S regulon devoted to transcription factors (8%); previously, σ^S was the only alternative σ factor in *E. coli* thought to control many transcription factors (64). The σ^{32} regulon contains several two-component systems and a number of transcriptional repressors, which together extend the reach of the regulon, sometimes in subtle ways. As examples, the two-component regulator PhoP/Q and the repressor TreR are transcribed by σ^{70} as well as by σ^{32} (28). Additionally, TreR is activated by PhoP/Q. PhoP/Q senses low Mg^{2+} and is induced by acid stress; dual control by σ's permits cells to have a greater response to acid stress at high temperature than at low temperature, possibly because combinations of stresses are more deleterious than single stresses. Likewise, the increased synthesis of TreR at high temperature by σ^{32} and by σ^{70} activated by PhoP/Q acts synergistically with σ^S to increase the accumulation of trehalose, which exerts both osmoprotective and heat protective effects on proteins and membranes. Trehalose synthesis is under σ^S control and goes through a trehalose phosphate intermediate. The σ^{32}-transcribed and PhoP/Q-activated TreR represses the pathway for degrading external trehalose, which is imported to the cell as trehalose phosphate (22). Increased TreR synthesis presumably ensures that all trehalose phosphate is shunted toward the production of trehalose.

Perhaps the most surprising realization concerning the role of the σ^{32} regulon was that many of its members (~25%) are either membrane localized or involved in membrane-relevant functions. Some relevant functions encoded by the regulon are carbonic anhydrase, necessary to provide the bicarbonate consumed in making fatty acids; components of the system for making disulfide bonds; enzymes involved in lipoprotein maturation; and membrane-localized histidine kinase sensors, transporters, and proteases. This suggests that a major function of the response is to maintain the integrity of the inner membrane upon heat stress (Fig. 5B). Interestingly, a global analysis of overproduced proteins that induce the HSR indicated that a disproportionate fraction of the inducers were membrane proteins (44). In this regard, it is intriguing that the housekeeping function of FtsH is the degradation of unassembled or damaged inner membrane proteins (24). Thus, the FtsH protease is responsible both for quality control of the membrane and for the degradation of σ^{32}. It remains to be determined whether the fluxes through each pathway allow the two processes to be coordinated.

The HSF-mediated HSR in eukaryotes, which is similar to the σ^{32}-mediated response in prokaryotes, has also been analyzed on the global level (18). Here too, many additional functions not related to protein homeostasis are encoded by the regulon. Interestingly, all regulatory loops currently known to control the HSR in both prokaryotes and eukaryotes are related to protein homeostasis. Two interpretations are possible. First, protein homeostasis may be the central function of the response. However, when protein homeostasis is upset, other cellular loops are likely to be perturbed as well. Therefore, the response has evolved to encompass these functions in addition to those involved in protein homeostasis. Alternatively, the response may be sensitive to numerous inputs, but only the regulatory responses related to protein homeostasis have been identified to date. In either case, the response is considerably broader than previously imagined.

EXTENDING THE LESSONS LEARNED FROM *E. COLI* TO OTHER ORGANISMS AND SYSTEMS

The lessons learned from the *E. coli* HSR can be applied to our understanding of heat shock and other stress responses in other systems. The HSR is universal, and some hsp's are among the most highly conserved proteins in the cell; however, σ^{32} homologues are present only in alpha-, beta-, and gamma-proteobacteria (41). While heat shock transcription factors are not as highly conserved, the regulation of the HSR appears similar in most organisms. The three most widespread HSR transcription factors are σ^{32}, HrcA, and HSF.

HSF is the eukaryotic heat shock transcription factor. HSF regulation is similar to σ^{32} regulation in many ways. HSF is a transcription factor that positively regulates the HSR. HSF is also directly negatively regulated by multiple chaperones, particularly Hsp70 (DnaK) and Hsp90 (reviewed in reference 43). As for *E. coli*, a sequestration model has been proposed to explain the mechanism of chaperone regulation of the transcription factor. However, as this model may not be sufficient for the regulation of σ^{32} in the simple *E. coli* system, it may be unlikely that this mechanism applies in the more complicated eukaryotic system. In addition to chaperone regulation, HSF can be regulated at many other levels, including but not limited to oligomerization, phosphorylation, and localization. However, due to the complexities of these regulations, it is often unclear how these components contribute to HSF regulation during stress.

The regulation of HrcA, a heat shock transcription factor that is widespread in bacteria, also has many similarities to σ^{32}, even though it functions as a transcriptional repressor. As is the case for the σ^{32}-mediated HSR, chaperones negatively regulate heat shock gene transcription in HrcA-dependent systems (34), albeit by a more complex route. Here, chaperones positively regulate HrcA because they are required for the proper folding of the protein. In turn, HrcA negatively represses heat shock gene transcription (reviewed in reference 42). However, it is not clear whether this system has a regulatory loop that directly responds to temperature that is comparable to what is seen for the translational regulation of σ^{32}. One interesting unexplored possibility is that the folding of HrcA is thermosensitive; in this case, HrcA itself might be a thermosensor. As temperature-dependent effects are present in other HSRs, it is likely that there are such effects in this system as well.

While much work has been done, further analysis of the HSR is critical. We have made good progress in understanding

some of the main regulators of the HSR, yet there are still as-yet-unknown components. Additionally, as these responses are universal and some components are highly conserved, HSRs provide an ideal situation, since analysis of the response in simple organisms is likely to be directly applicable to our understanding of the HSRs in general. HSRs are important for physiological processes such as cell signaling, a wide variety of pathological conditions, and evolution; therefore, expanding our understanding of the regulation of these responses is vital.

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