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The DnaK chaperone modulates the heat shock response of *Escherichia coli* by binding to the σ^{32} transcription factor

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ABSTRACT **The heat shock response and the heat shock proteins have been conserved across evolution. In *Escherichia coli*, the heat shock response is positively regulated by the σ^{32} transcriptional factor and negatively regulated by a subset of the heat shock proteins themselves. In an effort to understand the regulation of the heat shock response, we have purified the σ^{32} polypeptide to homogeneity. During the purification procedure, we found that a large fraction of the overexpressed σ^{32} polypeptide copurified with the universally conserved DnaK heat shock protein (the prokaryotic equivalent of the 70-kDa heat shock protein, HSP70). Further experiments established that purified σ^{32} bound to DnaK and that this complex was disrupted in the presence of ATP. Consistent with the fact that *dnaK756* mutant bacteria overexpress heat shock proteins at all temperatures, purified DnaK756 mutant protein did not appreciably bind to σ^{32}.**

All organisms respond to environmental stresses, such as heat and ethanol, by a rapid, vigorous, and transient acceleration in the rate of synthesis of a group of proteins collectively called the heat shock proteins (HSPs) (for reviews see refs. 1–4). Many of these HSPs have been conserved across evolution. For example, the DnaK and GroEL proteins of *Escherichia coli* are ≈50% identical, at the amino acid sequence level, to the eukaryotic HSP70 and HSP60 homologues (4), respectively. The *dnaJ* gene is another example of a heat shock gene that is highly conserved in evolution (5–8).

E. coli has ≈20 heat shock genes whose expression is positively regulated by the σ^{32} polypeptide [the *rpoH* (*htpR*) gene product] at the transcriptional level. σ^{32} is extremely unstable *in vivo*, with a half-life of ≈1 min at normal temperatures (9–11). In addition, the heat shock response of *E. coli* is negatively autoregulated. Specifically, mutations in either the *dnaK*, *dnaJ*, or *grpE* heat shock gene can lead to an overproduction of HSPs (10, 12, 13). Such overexpression of the heat shock response is due in part to a stabilization of the σ^{32} polypeptide in such genetic backgrounds (10, 11).

DnaK can bind to other polypeptides, such as GrpE, λP, p53, staphylococcal A protein, and unfolded bovine pancreatic trypsin inhibitor (14–19). Such binding is disrupted in the presence of hydrolyzable ATP. In addition, overproduction of DnaK aids the export of an otherwise export-deficient MalB–LacZ hybrid protein (20). DnaK, also referred to as a "chaperone" (21), is capable of promiscuously binding to various unfolded polypeptides, allowing them to (*i*) maintain the unfolded form and thus prevent misfolding, (*ii*) traverse biological membranes, (*iii*) be protected from aggregation or denaturation under conditions of stress, and (*iv*) be disaggregated when damaged by stress (reviewed in refs. 4, 22, and 23).

DnaJ and GrpE can function synergistically with DnaK—e.g., in the replication of bacteriophages λ and P1 (24–26). In addition, the ATPase activity of DnaK is greatly stimulated in the presence of both GrpE and DnaJ (18). Thus, at least part of the biological role of DnaJ and GrpE would be to facilitate the efficient "recycling" of DnaK (18).

Skelly *et al.* (27) showed that a small fraction of purified $E\sigma^{70}$ and $E\sigma^{32}$ RNA polymerase (RNAP) holoenzyme preparations was associated with DnaK, even after extensive purification. No specific function has been assigned to this putative DnaK–RNAP interaction. It could be that a small fraction of the RNAP molecules are in an aggregated form. Since DnaK can disaggregate such RNAP aggregates (23), it could associate with them during the purification procedure.

In this work we purified σ^{32} polypeptide to homogeneity and demonstrated that wild-type DnaK, but not DnaK756 mutant protein, could bind to σ^{32} polypeptide *in vitro*. Such a DnaK–σ^{32} association could potentially serve to negatively modulate heat shock gene expression either by sequestering σ^{32}, thus preventing it from binding to the RNAP core and forming $E\sigma^{32}$ holoenzyme, by actively stripping σ^{32} from $E\sigma^{32}$ (28), or by accelerating degradation of σ^{32} by presenting it to various proteases (3, 29). These possibilities are not mutually exclusive.

MATERIALS AND METHODS

Construction of an Inducible *rpoH*-Containing Plasmid. To obtain a stable plasmid construct that overproduced the σ^{32} polypeptide, the 1.3-kilobase (kb) *Eco*RV fragment from plasmid pFN97 (30) was cloned into the *Sma* I site of pTTQ18 (30, 31), to give rise to pTPG2. All known *rpoH* promoters have been removed in pTPG2 as a consequence of the cloning procedure. pTPG2 carries a *lacI*^{Q} gene, whose product binds to the upstream region and silences the expression of the cloned *rpoH* gene. Addition of isopropyl β-D-thiogalactopyranoside (IPTG) leads to LacI repressor inactivation and initiation of *rpoH* gene transcription.

Purification of σ^{32}. *E. coli* bacteria carrying pTPG2 were grown at 30°C to a density of ≈5 × 10^8 cells per ml in L broth (13) supplemented with ampicillin (50 μg/ml). At this point IPTG was added (1 mM) and growth was allowed to proceed for 1 hr. The 10-liter culture was harvested by centrifugation at 0°C in a Beckman JA-10 rotor for 15 min at 6000 rpm. The bacterial pellet was resuspended in 80 ml of buffer A [50 mM Tris·HCl pH 7.2/10% (wt/vol) sucrose/2 mM EDTA/1 mM dithiothreitol/230 mM NaCl/0.1% lysozyme/0.01% phenylmethylsulfonyl fluoride]. After a 30-min incubation on ice, sodium deoxycholate was added to a concentration of 0.05%, and the bacteria were lysed by sonication (three times for 0.5

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Abbreviations: HSP, heat shock protein; IPTG, isopropyl β-D-thiogalactopyranoside; RNAP, RNA polymerase.

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min at 70 W) at 0°C. The lysate centrifuged in a Beckman type 35 rotor at 25,000 rpm for 90 min at 0°C. Proteins were precipitated from the supernatant by ammonium sulfate (0.35 g/ml). The pellet was dialyzed for 16 hr against buffer B [50 mM Tris·HCl, pH 7.2/12 mM 2-mercaptoethanol/10% (vol/vol) glycerol/50 mM NaCl/0.5 mM EDTA] and loaded onto a Q-Sepharose column (Pharmacia, 2.6 × 6.5 cm) equilibrated with the same buffer. The adsorbed proteins were eluted with a 400-ml linear gradient of 50–400 mM NaCl in buffer B. Fractions containing σ^{32}, as judged by Western blot analysis (32) using anti-σ^{32} serum (generously provided by Herbert Weissbach, Hoffman–La Roche), were pooled and precipitated by addition of ammonium sulfate (0.35 g/ml). The pellet was dialyzed for 12 hr against buffer C [10 mM imidazole, pH 7.0/10% (wt/vol) sucrose/0.5 mM EDTA/5 mM 2-mercaptoethanol] with 100 mM NaCl and was loaded onto a heparin-agarose column (Sigma, 1 × 6 cm). Adsorbed proteins were eluted with a 100-ml linear gradient of 100–700 mM NaCl in buffer C. The σ^{32} protein was found in two peaks. The first was eluted at ≈300 mM NaCl, probably through its interaction with DnaK (see Fig. 2 and text). The second peak of σ^{32} was at ≈420 mM NaCl. Further purification was carried out only with material from the second peak. Fractions containing σ^{32} were pooled and dialyzed against buffer C with 50 mM NaCl and were loaded onto a P11 column (Whatman, 1.5 × 3 cm). The adsorbed proteins were eluted with a 100-ml linear gradient of 50–500 mM NaCl in buffer C. Fractions containing the most pure σ^{32} (as judged by SDS/PAGE and Coomassie blue staining), which was eluted at the beginning of the gradient, were pooled and applied directly to a hydroxyapatite column (Bio-Rad, 0.9 × 1.5 cm) equilibrated with buffer D [20 mM potassium phosphate, pH 6.8/100 mM NaCl/10% (wt/vol) sucrose/1 mM 2-mercaptoethanol]. Adsorbed proteins were eluted with a 20-ml linear gradient of 20–200 mM potassium phosphate in buffer D. Fractions containing pure σ^{32} were pooled and dialyzed against buffer E [40 mM Hepes·KOH, pH 7.6/10% (vol/vol) glycerol/0.1 mM EDTA/100 mM NaCl/1 mM 2-mercaptoethanol] and frozen in liquid nitrogen. σ^{32} activity is stable at −70°C for at least 6 months and can withstand repeated freezing and thawing.

Purification of Other Enzymes. DnaK and DnaK756 proteins (14) and RNAP core and σ^{70} (33) were purified as described. The purity of all enzymes used was >90%, except for σ^{70} (≈70%).

***In Vitro* Transcription.** Run-off transcription experiments were performed (34) with NaCl at 160 mM. The transcriptional activity of the σ^{32} protein in each fraction following filtration and glycerol gradient centrifugation experiments was measured by using pCG1 DNA, which carries an intact *dnaK* gene (13), as a template. The 25-μl reaction mixtures contained 20 mM Tris·HCl (pH 8.0), 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 200 mM NaCl, 0.16 mM each NTP (and 1 μCi of [^{3}H]UTP; Amersham; 1 μCi = 37 kBq), 1 μg of pCG1 DNA, 2.8 μg of RNAP core, and aliquots from each fraction. Reaction mixtures were incubated for 10 min at 37°C and the reaction was stopped as described by Chamberlin *et al.* (35). [^{3}H]UMP incorporated into trichloroacetic acid-insoluble material was estimated by liquid scintillation (35).

Glycerol Gradient Centrifugation. Reaction mixtures (100-μl) in buffer E (with 1 mM dithiothreitol instead of 2-mercaptoethanol) were assembled on ice, the order of addition of the proteins being σ^{32}, σ^{70}, DnaK, and finally RNAP core. The mixtures were incubated for 10 min at 37°C, and then loaded directly onto a 3.2-ml linear 15–35% (vol/vol) glycerol gradient and centrifuged at 2°C in a Beckman SW60 rotor for 25 hr at 48,000 rpm. Fractions were collected from the bottom of the tube and analyzed for transcriptional activity. The experiments of Fig. 5 were done with a linear 15–40% glycerol gradient, with centrifugation for 20 hr.

Size Chromatography. A P-200 column (Bio-Rad, 0.5 × 8 cm) was equilibrated with buffer F (40 mM Hepes·KOH, pH 7.6/100 mM NaCl/5 mM $MgCl_2$/1 mM 2-mercaptoethanol/0.02% Triton-X100) and 1 mM ATP, where indicated. Reaction mixtures (≈70 μl) containing the above-described proteins in buffer F were incubated for 10 min at 37°C and then loaded onto the P-200 column. Fractions of four drops each (≈100 μl) were collected. The presence of σ^{32} in each fraction was monitored by the transcription assay and by SDS/PAGE followed by Coomassie blue staining.

RESULTS

Purification of σ^{32}P. To investigate the mechanism of heat shock regulation, we purified the σ^{32} polypeptide. Up until this work, σ^{32} had been obtained by first purifying Eσ^{32} holoenzyme, separating the subunits by SDS/PAGE, removing the σ^{32} band, and renaturing it (34, 36). The *rpoH* gene was cloned under a strong inducible promoter, because overproduction of σ^{32} is not tolerated well by *E. coli* (11). σ^{32} was overproduced following IPTG induction and purified to homogeneity (Fig. 1*A*).

Purified σ^{32} Is Transcriptionally Active. To show that the purified σ^{32} was active in heat shock promoter recognition, *in vitro* run-off transcription experiments were performed. The *Nru* I–*Pvu* I DNA fragment containing both the P1 and P2 heat shock promoters of *dnaK* (38) was used as template. When only RNAP core was present, no significant transcription was obtained (Fig. 1*B*). Addition of purified σ^{32} allowed the reconstituted Eσ^{32} holoenzyme to initiate transcription at the appropriate sites, as judged by the length of the two run-off transcripts. Similar to the results of Cowing *et al.* (38), no transcription from the putative P3 heat shock promoter was observed.

DnaK Copurifies with σ^{32}. We observed an interaction between σ^{32} and DnaK during the purification of σ^{32}. After elution of proteins from the heparin-agarose column σ^{32} was

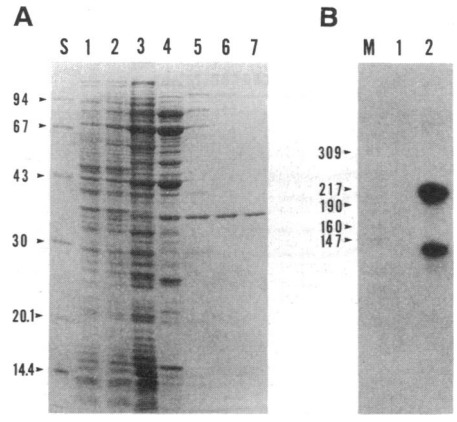

FIG. 1. (*A*) Successive steps in σ^{32} purification. SDS/12.5% PAGE of samples from the various purification steps. Lane 1, B178 carrying the pTTQ18 plasmid vector induced with 1 mM IPTG for 1 hr; lane 2: B178 carrying pTPG2 plasmid induced with 1 mM IPTG for 1 hr; lane 3, crude lysate after ammonium sulfate precipitation; lane 4, after Q-Sepharose chromatography; lane 5, after heparin chromatography; lane 6, after P11 chromatography; lane 7, after hydroxyapatite chromatography; lane S, molecular weight standards: phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,100), and α-lactalbumin (14,400). (*B*) *In vitro* transcription. Run-off transcripts were produced with the *Nru* I–*Pvu* I DNA fragment of the *dnaK* gene as templates. Lane 1, RNAP core; lane 2: RNAP core supplemented with purified σ^{32} polypeptide; lane M, DNA standards: pBR332 DNA digested with *Msp* I and end-labeled by using [γ-^{32}P]ATP and polynucleotide kinase (37). Only some of the fragments are visible. Lengths are indicated in bases.

found in two regions of the gradient (Fig. 2). Western blot analysis using both anti-σ^{32} and anti-DnaK antibodies showed that one region matched the peak of DnaK (300 mM NaCl) (Fig. 2 and data not shown). The DnaK/σ^{32} stoichiometry was not established at this point, because a protein similar in size to DnaK was present in fractions 1–3, and because it is difficult to quantitate with the staining and Western blotting procedures. The fact that in the absence of σ^{32} overproduction, DnaK is found exclusively in the void volume of the heparin-agarose column argues against a coincidental coelution of the two proteins. In agreement with this, the bulk of DnaK from the fractions loaded onto the heparin column was also found in the excluded volume.

When σ^{32} was overproduced and purified from isogenic *dnaK756* mutant bacteria, <5% of either the σ^{32} or the DnaK756 protein was eluted at the first peak position (data not shown). This result also supports the conclusion that the DnaK–σ^{32} interaction is not fortuitous and that the interaction changes the chromatographic properties of both proteins in the heparin-agarose column.

To further confirm that authentic σ^{32} was present in both peaks, the $M_r \approx 32{,}000$ polypeptides were sequenced. In both instances the same N-terminal sequence (Thr-Asp-Lys-Met-Glu) was found, matching perfectly the one predicted by the DNA sequence (the first methionine being processed). Additionally, the transcriptional specific activities (see below), as well as the isoelectric points of the σ^{32} found in the two peaks, were identical (data not shown), suggesting that the two σ^{32} species were the same polypeptide.

DnaK and σ^{32} Form a Physical Complex. To determine whether DnaK and σ^{32} directly interact with each other, gel filtration and glycerol gradient centrifugation were performed. First, the two purified proteins were incubated together for 10 min at 37°C and then filtered on a small P-200 column. The presence of σ^{32} in the various fractions was monitored by the transcription assay and by SDS/PAGE. In all cases the position of the activity coincided perfectly with the position of σ^{32}. In addition, DnaK, at the levels used, did not significantly influence the σ^{32} transcriptional activity (data not shown).

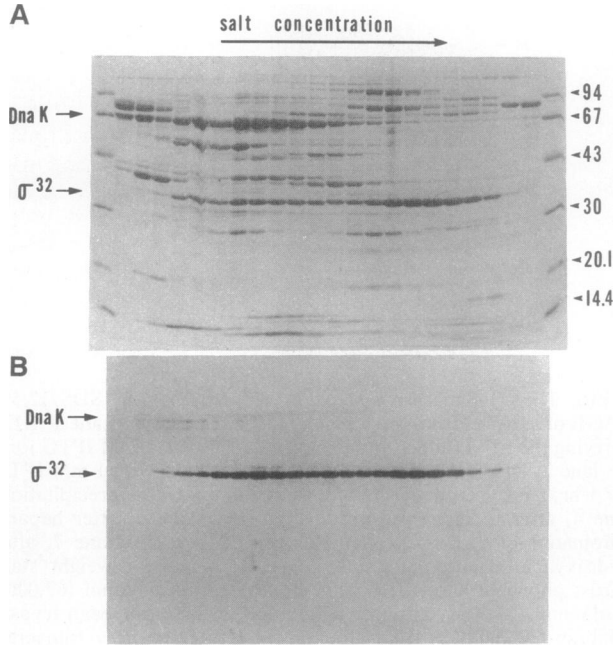

FIG. 2. Purification of σ^{32} on a heparin-agarose column. (*A*) SDS/12.5% PAGE following elution from the heparin column. (*B*) Western blot analysis with anti-σ^{32} antibodies of the same heparin gradient samples shown in *A*. The molecular weight standards shown at the ends are those described in the legend to Fig. 1*A*.

The σ^{32} polypeptide alone was eluted in fractions 9–12 (Fig. 3). However, when σ^{32} was incubated with DnaK (1:2.6 molar ratio), it was eluted in fractions 5–8, indicating its presence in a larger complex. From its activity in the transcription assay, and its physical presence following SDS/PAGE, >80% of σ^{32} was found in a complex with DnaK (Fig. 3). When the DnaK–σ^{32} interaction was monitored in the presence of 1 mM ATP, σ^{32} was eluted in fractions 7–11, clearly an intermediate position between that of free σ^{32} and the DnaK–σ^{32} complex. Although the reasons for this chromatographic behavior are not known, an analogous result was observed for the DnaK–λP interaction in the presence or absence of ATP (figure 3*B* of ref. 17). One possibility is that continuous cycles of releasing and rebinding σ^{32} or λP by DnaK result in the observed intermediate position.

Equivalent results were obtained from glycerol gradient sedimentation. Even under such conditions, known to interfere with hydrophobic interactions, ≈40% of the σ^{32} activity was found in a complex with DnaK (Fig. 4). However, when ATP was present throughout the gradient, the DnaK–σ^{32} complex was not observed (Fig. 4).

The DnaK–σ^{32} interaction was also monitored by the ability of anti-DnaK antibodies to coprecipitate σ^{32}. Addition of RNAP core to the σ^{32}/DnaK incubation mixture abolished the coprecipitation of σ^{32} and DnaK (data not shown; see below).

DnaK756 and σ^{32} Do Not Form a Detectable Complex. *dnaK756* mutant bacteria overproduce HSPs at all temperatures tested and, in addition, fail to turn off the heat shock response at high temperatures (12). The DnaK756 mutant protein was purified and its association with σ^{32} was tested as described above. With both methods, no interaction whatsoever was observed between the two proteins (Figs. 3 and 4). We conclude that if an interaction between σ^{32} and DnaK756 exists, it must be unstable.

Competition Experiments with RNAP Core. Since σ^{32} interacts with both DnaK and the RNAP core, we performed experiments to determine the relative affinities of σ^{32} for either DnaK or RNAP core. Purified σ^{32} was incubated with either RNAP core, DnaK, or both for 10 min at 37°C. The interaction of σ^{32} with the other proteins was monitored by glycerol gradient centrifugation. The position of σ^{32} was

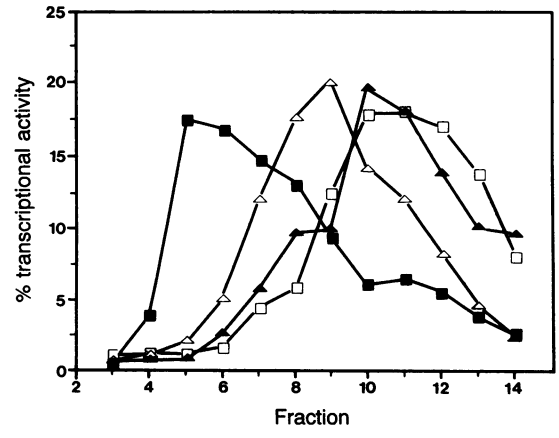

FIG. 3. Transcriptional activity of σ^{32} following incubation with DnaK and filtration on a P-200 column. ■, 2.4 μM σ^{32} incubated with 6.1 μM DnaK; □, 2.4 μM σ^{32} incubated alone; ▲, 2.4 μM σ^{32} incubated with 6.1 μM DnaK756; △, 2.4 μM σ^{32} incubated with 6.1 μM DnaK in the presence of 1 mM ATP (ATP was also present in the filtration buffer). The percent activity was calculated as the number of acid-precipitable counts in a particular fraction compared with the counts present in all fractions.

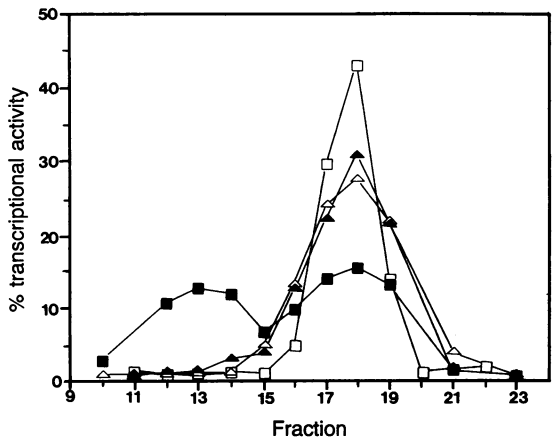

FIG. 4. Cosedimentation of σ^{32} with DnaK. Transcriptional activity was determined following incubation of σ^{32} protein with DnaK and sedimentation in a 15–35% glycerol gradient (SW60 rotor at 48,000 rpm at 2°C for 25 hr). ■, 1.7 μM σ^{32} incubated with 4.3 μM DnaK; □, 1.7 μM σ^{32} incubated alone; ▲, 1.7 μM σ^{32} incubated with 4.3 μM DnaK756; △, 1.7 μM σ^{32} incubated with 4.3 μM DnaK in the presence of 1 mM ATP.

established by the Western blot technique using ^{125}I-labeled staphylococcal A protein. Under such conditions, RNAP core has a much higher affinity for σ^{32} than does DnaK (Fig. 5). Even in the presence of an 8-fold molar excess of DnaK, we were unable to detect an appreciable DnaK–σ^{32} complex in the presence of RNAP core. However, when the σ^{32} and DnaK proteins were preincubated for 20 min at 42°C, and then RNAP core was added, a small amount of σ^{32} was found to still associate with DnaK (5–10% of total σ^{32}; data not shown).

Because of the potential antagonistic roles of σ^{32} and the "housekeeping" σ^{70} polypeptide in the regulation of the heat shock response, we also determined their relative affinities for the RNAP core enzyme. When both σ^{32} and σ^{70} (1:1 molar ratio) were present in the reaction, ≈40% of σ^{32} was found in a complex with RNAP core (Fig. 5). When DnaK was also added to the reaction mixture, the free σ^{32} was found in a DnaK–σ^{32} complex (Fig. 5). Similar results were obtained

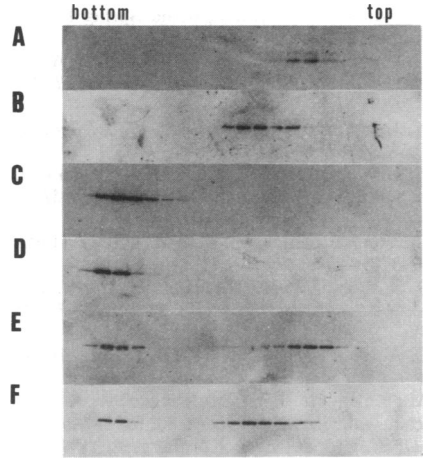

FIG. 5. Association of σ^{32} protein with RNAP core. Approximately 1 μM σ^{32} was incubated alone (*A*), with 9 μM DnaK (*B*), with 1.2 μM RNAP core (*C*), with 1.2 μM RNAP core and 9 μM DnaK (*D*), with 1.2 μM RNAP core and 1.1 μM σ^{70} (*E*), or with 1.2 μM RNAP core, 1.1 μM σ^{70}, and 9 μM DnaK (*F*). After these incubations, each mixture was sedimented in a 15–40% glycerol gradient (SW60 rotor at 48,000 rpm at 2°C for 20 hr). Fractions were collected from the bottom of each tube. The presence of σ^{32} was determined by Western blot analysis using ^{125}I-labeled staphylococcal A protein.

when the σ^{70}/σ^{32} competition was monitored through the ability of RNAP–σ^{32} holoenzyme to initiate transcription at the *dnaK* heat shock promoters. When σ^{70} was preincubated in equimolar amounts to σ^{32} with RNAP core, the transcription from the *dnaK* promoters was limited to 30% of the control (data not shown).

DISCUSSION

The classic heat shock response in *E. coli* is under the positive transcriptional regulation of the σ^{32} polypeptide and the negative regulation of the HSPs themselves (reviewed in refs. 1, 3, and 28). Expression of *rpoH* (*htpR*), coding for σ^{32}, is regulated by a variety of intricate transcriptional and posttranscriptional mechanisms (3). The transcriptional mechanisms include the presence of multiple promoters, which are regulated by temperature, state of supercoiling, catabolite state, and the DnaA DNA replication initiation protein of *E. coli* (3, 39–43). Posttranslational regulation includes cis-acting mRNA sites, which impede translation at low temperatures (44), as well as the extremely short half-life of σ^{32} (9–11). The half-life of σ^{32} is increased in *dnaK*$^-$, *dnaJ*$^-$, or *grpE*$^-$ genetic backgrounds, consistent with the fact that these mutations lead to an overproduction of HSPs (10–13). A possibility is that the σ^{32} polypeptide is a better substrate for proteases when complexed with DnaK protein (29). All of the above factors probably act synergistically to limit the intracellular level of σ^{32} to 10–30 copies per cell (45).

DnaK has been purified to homogeneity and has been shown to possess a number of interesting properties: (*i*) a very weak ATPase activity (46); (*ii*) an autophosphorylating activity (46); (*iii*) the ability to bind other proteins, including λO, λP, GrpE, and unfolded bovine pancreatic trypsin inhibitor (14, 17, 19, 28); (*iv*) the ability to protect RNAP–σ^{32} holoenzyme from heat inactivation (23); (*v*) the ability to disaggregate certain protein aggregates, such as RNAP (23), DnaA (47), and the multiprotein complex assembled at the origin of λ DNA replication (24, 48); (*vi*) the ability to suppress, when overproduced, the transport defect of a mutant LamB–LacZ hybrid polypeptide (20); (*vii*) the ability to disaggregate, with the help of DnaJ, RepA dimers into monomers (49); (*viii*) the ability to undergo a dramatic conformational change upon NTP hydrolysis (19); and (*ix*) a great stimulation of its ATPase activity in the presence of DnaJ and GrpE (18). These results have led to the following model (4, 28, 43): DnaK can bind promiscuously to many unfolded and some folded polypeptides. Such binding maintains these polypeptides in a form competent for either export across a membrane, proteolysis by some proteases, or proper folding/assembly. The polypeptides are released following ATP hydrolysis. In light of this model, protein disaggregation would result from multiple rounds of such binding and release by DnaK.

The purification of σ^{32} by classic biochemical techniques enabled us to demonstrate its direct protein–protein interaction with both DnaK and the RNAP core. The DnaK–σ^{32} interaction was observed (*a*) during σ^{32} purification, with approximately half of the σ^{32} population found associated with DnaK, and (*b*) between the two purified proteins in gel filtration and glycerol gradient sedimentation experiments, with the complex being disrupted in the presence of ATP. That 50% of the overproduced σ^{32} was found associated with DnaK and not with other major chaperones, such as GroEL, suggests a specific DnaK–σ^{32} interaction. This suggestion is further supported by the failure of the mutant DnaK756 protein to bind purified σ^{32}. However, σ^{32} associated with RNAP core much more readily than it did with DnaK; i.e., in the presence of an 8-fold molar excess of DnaK over RNAP core, purified σ^{32} still quantitatively associated with RNAP. The observation that, during purification, approximately half

of the overproduced σ^{32} protein entered into an association with DnaK suggests that *in vivo* the σ^{32} polypeptide may not have ready access to the RNAP core, perhaps because of the presence of σ^{70} and other factors that may associate with the RNAP core.

Our data are consistent with the following heat shock regulation model. At low temperatures, such as 30°C or below, and in the absence of protein-damaging conditions, DnaK binds to and effectively sequesters the majority of the σ^{32} molecules, thus damping the expression of heat shock genes, including *dnaK*. Since the two major *dnaK* promoters are transcribed exclusively by σ^{32} (ref. 38 and this work), its rate of transcription must be exquisitely regulated by the intracellular levels of both σ^{32} and DnaK. When *E. coli* undergoes a heat shock or encounters a protein-damaging agent, the levels of denatured, unfolded, and aggregated proteins increase. DnaK binds to such polypeptides, thus protecting them from further damage or disaggregating some of them (23). This leaves σ^{32} free to associate with RNAP core, leading to an increase in heat shock gene expression. When the level of damaged proteins subsides and/or the intracellular level of DnaK rises due to its accelerated rate of synthesis, the DnaK protein can resume its σ^{32}-sequestering "chores," thus interfering with its access to RNAP core and accelerating its destruction by proteases. The resumed DnaK–σ^{32} interaction will again result in damping of heat shock gene expression.

The demonstrated DnaK–σ^{32} interaction also helps explain the down-regulation of the heat shock response at low temperatures (50); when bacteria undergoing a heat shock response at 42°C are shifted back to 30°C, the expression of the heat shock response is transiently repressed. The excess DnaK protein synthesized at 42°C will be free to efficiently sequester the excess σ^{32} polypeptide at 30°C, thus damping the heat shock response. It would not be surprising to find that eukaryotes have evolved a similar HSC70/HSP70 "sequestration" mechanism of the heat shock factor (HSF) in order to autoregulate their heat shock response (45).

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