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Biochemistry

Heat shock of *Escherichia coli* increases binding of dnaK (the hsp70 homolog) to polypeptides by promoting its phosphorylation

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ABSTRACT **The "molecular chaperone", dnaK, is induced in *Escherichia coli* upon heat shock and promotes ATP-dependent refolding or degradation of damaged proteins. When cells were grown at 25°C and disrupted, a small fraction of the dnaK bound to affinity columns containing unfolded polypeptides (e.g., a fusion protein named CRAG or casein) and could be dissociated by ATP-Mg^{2+}. After shifting cells to 42°C for 30 min, up to 5-fold more dnaK bound to these columns than after growth at 25°C. This enhanced binding capacity was reversed after shifting cells back to 25°C. It resulted from a covalent modification, which decreases dnaK's electrophoretic mobility and isoelectric point. This modification appears to be phosphorylation; after treatment with phosphatases, the ATP-eluted dnaK resembled the predominant form in electrophoretic and binding properties. In addition, after incubating cells with [^{32}P]orthophosphate at 42°C, the ^{32}P-labeled dnaK bound quantitatively to the CRAG column, unlike the nonlabeled protein. Thus, the phosphorylated dnaK is a special form of the chaperone with enhanced affinity for unfolded proteins. Its accumulation at high temperatures may account for dnaK's function as the "cellular thermometer."**

When bacterial or eukaryotic cells are exposed to high temperatures (>42°C) or to other harsh conditions (e.g., oxidative stress or heavy metals), they synthesize a set of proteins called heat shock proteins (hsps) (1). These stress proteins appear to protect the cell against thermal injury and other toxic insults (2, 3). Members of the hsp70 and hsp60 families, called "molecular chaperones" (4), can facilitate the transport of proteins across cell membranes, prevent misfolding of damaged polypeptides, and promote their refolding and assembly into multimers through ATP-dependent processes (5–7). In eukaryotes, hsp70 and its homolog in the endoplasmic reticulum, BiP, bind to newly synthesized polypeptides as they are released from ribosomes (8) and thus appear to be critical in the normal folding of cytosolic or secretory proteins. The homolog of hsp70 in *Escherichia coli*, dnaK, is essential for cell survival at high temperatures (3, 9). It is an abundant protein, but upon heat shock, its level increases severalfold (3). *In vivo*, dnaK can promote the refolding of certain denatured proteins (10, 11) and is also essential for the rapid degradation of many abnormal proteins (12, 13). Recent findings suggest that if this chaperone fails to promote proper folding, it can facilitate the hydrolysis of the unfolded polypeptide.

Because dnaK and the other major hsp, groEL, appear to bind selectively to unfolded regions of proteins, we developed a one-step method to purify these chaperones and to follow their interactions with substrates (14). This approach uses as an affinity ligand a fusion protein, named CRAG (15), that contains short unfolded domains at either end of a truncated staphylococcal protein A, which is functional and binds tightly to immunoglobulins. When expressed in *E. coli*, CRAG is found associated with dnaK, groEL, and several minor proteins (grpE) (14, 15). If normal *E. coli* extracts are passed through a column containing CRAG, a fraction of the cell's groEL and dnaK binds to the CRAG at different sites. Low concentrations of ATP release the dnaK selectively, whereas the release of the groEL from CRAG requires higher concentrations of ATP or acid (14).

One unexpected finding was that the ability of groEL to dissociate from CRAG depended on the growth temperature of the bacteria (16). In extracts of cells that had been subjected to a brief heat shock, groEL could be released from CRAG efficiently by ATP even in the absence of its usual cofactor, groES. These altered dissociation properties are due to the phosphorylation of groEL at high temperatures (16). These findings suggested that reversible phosphorylation of chaperones might be an important feature of the heat-shock response that has major biological consequences. We demonstrate here that at high temperature, the other major hsp, dnaK, also undergoes a postsynthetic modification that alters its binding to unfolded proteins. This change in dnaK function is also due to reversible phosphorylation; however, this modification of dnaK promotes its binding to certain proteins, in contrast to groEL phosphorylation, which facilitates groEL release. Phosphorylation of dnaK has been noted before, both *in vitro* and *in vivo*, but no functional consequences were found (17, 18). Such heat-induced changes in dnaK's properties are of particular interest, since dnaK also seems to function as the "cellular thermometer" (18, 19).

MATERIALS AND METHODS

Bacterial strains were kindly provided by H. Hellebust (Royal Technology Institute, Stockholm), RR1Δm15 pRIT2, and by J. Beckwith (Harvard Medical School), MPH86 *lac*(am) *trp*(am) *mal*(am) *aro supC phoA61*. Cells were grown, proteins were labeled with tran^{35}S-label, and extracts were prepared as described (16).

The CRAG column was prepared as described (17). The ^{35}S-labeled cell extract (1–2 × 10^6 cpm/μg; 50 μg) was applied to 2 ml of the CRAG column. The column was washed with buffer (14) until the eluate contained less than 5–8 × 10^3 cpm/ml; 6 ml of buffer containing 1 mM ATP was then added to elute bound chaperones. The material that remained bound to the column after washing with 25 ml of this buffer was eluted with 100 mM acetic acid (pH 2.5). The ATP and acid-eluted proteins were precipitated with 10% (wt/vol) trichloroacetic acid (TCA), washed with acetone, and fractionated by SDS/PAGE (20) or by two-dimensional (2D) electrophoresis (21).

For labeling with [^{32}P]orthophosphate, MPH86 cells were grown overnight in low-phosphate medium (22), and an inoculum was then grown until midlogarithmic phase at 37°C.

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Abbreviations: hsp, heat shock protein; TCA, trichloroacetic acid; 2D, two dimensional.

The bacterial suspension (10 ml) was transferred to 25°C, and 1 mCi of [^{32}P]orthophosphate (Amersham; 1 Ci = 37 GBq) was added. Cells were incubated at 25°C for 3 h, shifted to 42°C for 1 h, harvested, and resuspended in 200 μl of buffer (20 mM Tris·HCl, pH 8.0/1 mM $MgCl_2$). After disruption by sonication, the extracts were treated with RNase (10 μg/ml) and DNase (10 μg/ml) on ice for 30 min.

RESULTS

When an extract of *E. coli* growing at 25°C was loaded onto the CRAG column, a small fraction of the dnaK bound strongly to it, and nearly all the bound dnaK could be eluted with ATP-Mg^{2+} (14). To quantitate the portion of the total cellular dnaK that bound and was eluted with ATP, we performed serial dilutions of the extracts, subjected them to SDS/PAGE, and analyzed them on Western blots using an anti-dnaK antibody and ^{125}I-labeled protein A. In parallel, we performed a Western blot analysis of the ATP-eluted dnaK and then calculated the relative amounts of the dnaK in these two samples. With an extract of *E. coli* grown at 25°C, only ≈2% of the total cellular dnaK bound to the CRAG column, and all of it was released upon ATP addition (14). In contrast, when growing cells were switched to 42°C for 30 min (i.e., to conditions that induce hsps), 4- to 5-fold more dnaK bound and was eluted with ATP (Fig. 1). However, under these conditions, the total amount of dnaK in the cell increased only 1.5- to 2-fold. Thus, heat shock seems to enhance the ability of some of the cell's dnaK to bind to CRAG at least 2- to 3-fold. In different experiments, the fraction of the cell's dnaK that associated with CRAG varied; however, in each case, there was a 4- to 5-fold enhancement of binding after incubation at 42°C for 30 min. Longer exposure of cells to 42°C further increased the fraction of dnaK that bound to CRAG, and after 1 h, 7- to 8-fold more dnaK bound to CRAG and was released by ATP than at 25°C.

To test whether this change in binding was permanent, cells growing at 25°C were shifted to 42°C for 30 min and then returned to 25°C. During the subsequent incubation at 25°C, the amount of dnaK that bound to CRAG decreased by ≈50% in 30 min, even though the total amount of dnaK in the culture did not fall. After the heat shock, the cell's dnaK content was about twice the level seen during the initial growth at 25°C. Thus, after returning cells to 25°C for 30 min, the fraction of the cellular dnaK that bound to CRAG and was eluted with ATP was similar to that before the heat shock. The heat-induced increase in dnaK-binding capacity is, therefore, fully reversed upon down-shift of the growth temperature. A very similar increase in dnaK binding after heat shock of cells and reversal at 25°C was also observed when denatured casein was used as the affinity ligand on the column instead of CRAG (data not shown). Thus, heat shock appears to generally enhance the capacity of dnaK to bind to abnormal proteins.

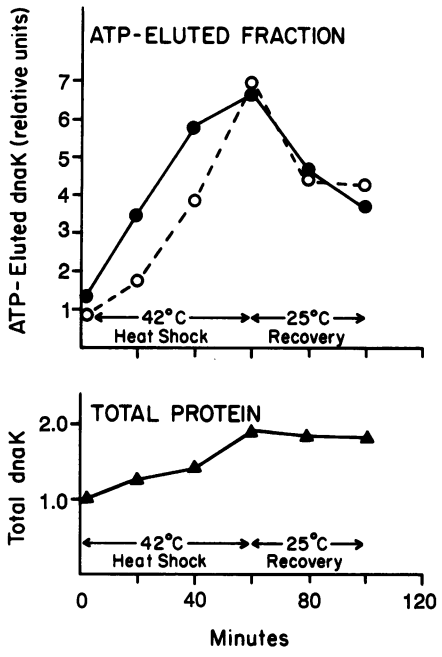

FIG. 1. Effect of heat shock (42°C) and recovery at 25°C on the binding of dnaK (○) and groEL (●) to CRAG column. Relative amounts of dnaK and groEL in the ATP-eluted fractions (*Upper*) and in total cell extract (*Lower*) were determined with anti-dnaK and anti-groEL antibodies.

This increase in binding upon heat shock must be due to some chemical alteration in dnaK and not just to an increase in the cell's content of dnaK. Accordingly, when cells were shifted to 42°C in the presence of chloramphenicol at 0.1 mg/ml to block protein synthesis, heat shock still caused a 2-fold increase in the amount of dnaK that bound and was eluted by ATP from the CRAG column (Table 1). Thus, heat shock not only induces the synthesis of dnaK but also enhances the binding of the preexistent dnaK to CRAG, probably by causing a postsynthetic alteration of the chaperone.

We have shown (16) that upon heat shock of *E. coli*, some of the cell's groEL undergoes a covalent modification that facilitates its dissociation from CRAG. To test whether these changes in the behavior of dnaK and groEL may in some way be linked, we compared the time courses of the changes in their properties upon heat shock and return to 25°C (Fig. 1). During incubation at 42°C, the amounts of dnaK and groEL eluted with ATP increased linearly at roughly similar rates. Upon down shift to 25°C, the time course of the changes in the properties of dnaK and groEL was also very similar. Thus, heat shock appears to affect simultaneously the capacity of both chaperones to bind to or be released from unfolded proteins.

To define the basis for the increased binding capacity of dnaK after heat shock, the mobilities of the ATP-eluted form and of the major fraction of dnaK in the cell were compared by SDS/PAGE. The apparent molecular weight of the dnaK eluted from the CRAG column was 1–2 kDa higher than that of the predominant dnaK band (Fig. 2*A*). Both bands reacted with anti-dnaK antibody with equal affinities. Since the protein was denatured by boiling in SDS and 2-mercaptoethanol prior to electrophoresis, this difference in mobility indicates that the ATP-eluted form is covalently modified. Furthermore, upon 2D isoelectrofocusing/SDS/PAGE, the ATP-eluted dnaK showed a more acidic isoelectric point than the major form (Fig. 2 *B* and *C*).

The heat-modified form of dnaK was converted rapidly to the predominant form after elution from the CRAG column. When this modified dnaK was incubated at 4°C for 4 h, the band of 72 kDa shifted to 70 kDa, the size of the unmodified dnaK (Fig. 3). The addition of 2% (wt/vol) SDS, boiling, or freezing at −70°C prevented this demodification (data not shown). Thus, this change in mobility appears to be due to an enzymatic activity, which is an inherent activity of dnaK or copurifies with dnaK off the CRAG column. In either case,

Table 1. Heat shock stimulates binding of dnaK to CRAG in the presence of chloramphenicol

Incubation for 30 min	ATP-eluted dnaK, arbitrary units		
	Exp. I	Exp. II	Exp. III
At 25°C	0.32	0.30	0.12
At 42°C + chloramphenicol	0.60	0.55	0.20

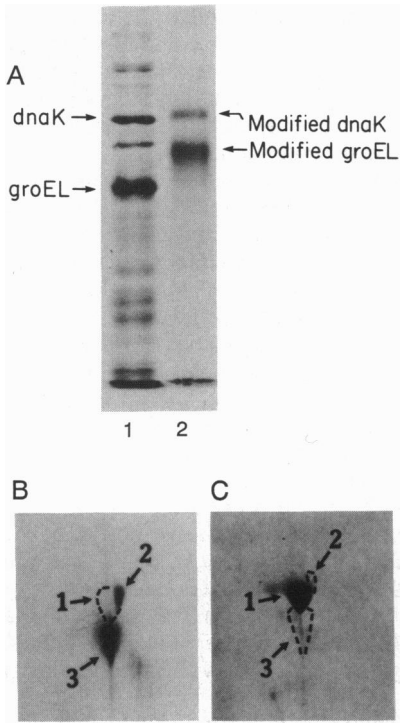

FIG. 2. (*A*) ATP-eluted fraction of dnaK has a different apparent molecular weight than the major form of dnaK. The ATP-eluted fraction and the total extract from the heat-shocked cells were separated by SDS/PAGE on 7.5% gels. Lanes: 1, total cell extract; 2, ATP-eluted fraction. (*B* and *C*) Comparison of the mobilities of modified and predominant forms of dnaK by 2D electrophoresis. Unlabeled dnaK protein purified by conventional methods with ATP-agarose (14) [kindly provided by C. Georgopoulos (University of Geneva)] was mixed with the radiolabeled fraction eluted with ATP from the CRAG column and subjected to 2D electrophoresis. Thus, the mobilities of the two forms of dnaK were compared on the same gel. (*B*) Autoradiography. (*C*) Coomassie blue staining. Spots: 1, unmodified dnaK; 2, modified dnaK; 3, modified groEL. The 2D electrophoresis was performed as described by Phillips *et al.* (21).

this enzymatic demodification of dnaK may account for the reversal of the heat-induced properties seen *in vivo* upon return to 25°C.

Several observations suggested that the modification that enhances dnaK binding might be phosphorylation. Heat shock alters the properties of groEL under the same conditions (Fig. 1) by inducing its phosphorylation (16). Also, Zylicz *et al.* (17) showed that purified dnaK possesses an autokinase activity, whose functional consequences and physiological significance are not clear. Cells were therefore labeled at 42°C in low phosphate medium with [^{32}P]orthophosphate. The prelabeled extracts were loaded onto the CRAG column, and dnaK was eluted with nonradioactive ATP and subjected to SDS/PAGE. Autoradiography of these fractions revealed that the ATP-eluted form of dnaK had incorporated ^{32}P *in vivo* (Fig. 4). Accordingly, S. Farr (personal communication) has found by 2D electrophoresis that heat shock of *E. coli* markedly stimulates dnaK phosphorylation.

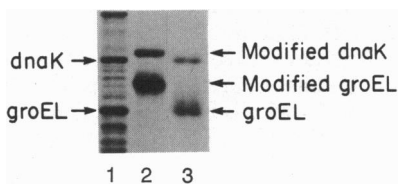

FIG. 3. Disappearance of the modified form of dnaK upon incubation at 4°C after elution from CRAG. Storage of the ATP-eluted fraction of dnaK for 4 h at 4°C caused a shift in its apparent molecular weight to a form resembling that of the major dnaK fraction. Lanes: 1, total extract of heat-shocked cells; 2, fraction eluted from CRAG with ATP; 3, same ATP-eluted fraction after incubation for 4 h at 4°C.

To determine whether phosphorylation is responsible for the heat-induced changes in dnaK's binding properties, ^{35}S-labeled dnaK eluted with ATP from the CRAG column was treated with alkaline phosphatase prior to SDS/PAGE. This treatment caused a shift of the band of the modified dnaK to the size corresponding to the unmodified form (Fig. 5). To test whether the phosphorylated dnaK associated selectively with CRAG, we measured the fraction of the phosphorylated chaperone bound to the column. Growing cells were labeled at 42°C with [^{32}P]orthophosphate. One-half of this extract was precipitated with TCA; the other half was loaded onto the CRAG column and the flow-through was collected and precipitated with TCA. Both samples were treated with TCA to block phosphatase activities and to prevent dnaK dephosphorylation during the prolonged incubation with antibodies and protein A-Sepharose. Since loading of the extract onto the column and collecting the flow-through took ≈5 min, dephosphorylation must be insignificant (Fig. 3). The proteins in these samples were solubilized (23), and dnaK was immunoprecipitated with anti-dnaK antibody.

As expected, the amount of dnaK that could be isolated from the extract with the anti-dnaK antibody (measured by Coomassie blue staining) was similar to the amount in the flow-through of the CRAG column (i.e., as discussed above, only a small percent of the total cellular dnaK bound to the column). Nevertheless, autoradiography demonstrated a clear phosphorylated band in the extract, even though no ^{32}P was found associated with the dnaK in the flow-through fraction (Fig. 6). In other words, all of the cell's phosphorylated dnaK had become bound to the CRAG column. Although purified dnaK (17, 18) can undergo ATP-dependent autophosphorylation, the quantitative recovery from the CRAG column of phosphorylated dnaK (Figs. 4 and 6) cannot be explained by phosphorylation during ATP-induced release from the column. The ^{32}P incorporation into the chaperone must have occurred *in vivo*, since the extracts contained 2.5 mM EDTA, which blocks autophosphorylation, whereas elution was with nonradioactive ATP. Moreover, immunoprecipitation with anti-dnaK antibody from the ^{32}P-labeled extract and from the flow-through of the column indicated that dnaK phosphorylated *in vivo* binds to CRAG selectively and quantitatively, unlike the predominant species.

To investigate directly the effect of dephosphorylation on dnaK's binding capacity, the cell extract was treated with alkaline phosphatase for 15 min before loading onto the CRAG column. This treatment decreased the amount of dnaK recovered in the ATP-eluted fraction severalfold (Fig. 7*A*). Thus, phosphorylation clearly was important for binding. We also studied the effect of dephosphorylation of purified dnaK on its binding properties. dnaK was eluted from the CRAG column with ATP in the presence of 1 mM $MgCl_2$. This material was divided into two parts, one of which

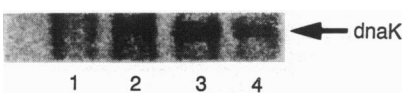

FIG. 4. Heat-shock-modified form of dnaK is phosphorylated. Cells were shifted to 42°C for 40 min in low phosphate medium in the presence of [^{32}P]orthophosphate. Extracts from labeled cells were passed through the CRAG column, and the dnaK was eluted with ATP. Lanes: 1, eluate before ATP addition; 2–4, sequential fractions (1 ml) eluted with ATP. High background reflects the presence of undigested fragments of DNA or RNA highly labeled with ^{32}P.

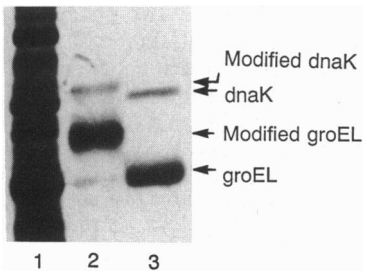

FIG. 5. Effect of alkaline phosphatase treatment on the mobility of the modified form of dnaK by SDS/PAGE. After ATP elution of the heat-modified dnaK from the CRAG column, 1 mM $ZnCl_2$ and 10 units of alkaline phosphatase from calf intestine (Boehringer Mannheim) were added. This mixture was incubated for 15 min at 37°C, precipitated with TCA, and subjected to SDS/PAGE to detect a dnaK band shift. Lanes: 1, total extract of cells after heat shock (42°C); 2, ATP-eluted fraction; 3, same fraction after treatment with the phosphatase.

was treated with alkaline phosphatase. After a 15-min incubation, the eluted samples were reloaded onto the CRAG columns in the presence of 10 mM EDTA (to prevent ATP hydrolysis and immediate dissociation of the dnaK–CRAG complex). After dilution, this protein rapidly lost its binding ability for unknown reasons, and after 15 min only ≈20% of the dnaK bound again to the column. However, after phosphatase treatment, four times less dnaK (≈5%) became bound to CRAG than with the control eluate (Fig. 7*B*). Thus, the phosphorylation of dnaK during heat shock was responsible for its greater binding to the denatured protein.

DISCUSSION

These findings on dnaK and related ones on groEL (16) demonstrate an important aspect of the heat-shock response: that, in addition to the dramatic changes in gene transcription at high temperatures, a fraction of the major hsps undergoes reversible phosphorylation, which alters their interactions with target proteins. This modification markedly enhances the capacity of dnaK to bind to unfolded proteins but may

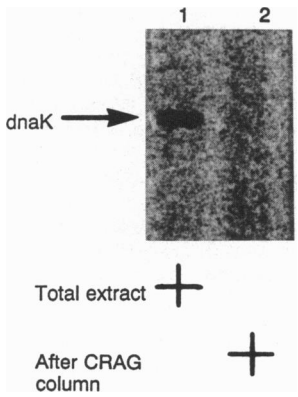

FIG. 6. Phosphorylated form of dnaK quantitatively binds to the CRAG column. ^{32}P-labeled cell extract was loaded onto the CRAG column, and the dnaK in the total cell extract and in the flow-through was precipitated with the anti-dnaK antibody. For immunoprecipitation of dnaK, the ^{32}P-labeled cell extract and the flow-through from the CRAG column were precipitated with 10% TCA. Pellets were washed twice with acetone and solubilized in 6 M urea/0.5 M 2-mercaptoethanol/0.3% SDS. Dissolved proteins were diluted 1:50 with phosphate-buffered saline containing 1% Triton X-100 and incubated with the specific antibodies and protein A-Sepharose. Autoradiography of the immunoprecipitates was performed after the SDS/PAGE. Lanes: 1, precipitate obtained with anti-dnaK antibody from the total cell extract; 2, same from flow-through.

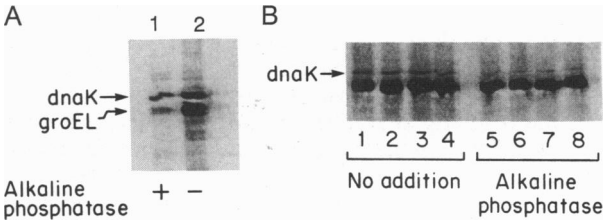

FIG. 7. (*A*) Alkaline phosphatase treatment of cell extract inhibits the binding of dnaK to CRAG. ^{35}S-labeled cells were harvested, resuspended in 50 mM Tris·HCl (pH 8.0) containing 1 mM $MgCl_2$, and disrupted by sonication. The extract was incubated with alkaline phosphatase in presence of 1 mM $MgCl_2$ at 37°C for 15 min, then 2.5 mM EDTA was added, and the extract was loaded onto the CRAG column. dnaK and groEL were recovered by ATP elution. Lanes: 1, extract treated with the phosphatase; 2, control extract incubated in parallel, but without alkaline phosphatase. (*B*) Treatment with alkaline phosphatase inhibits the binding of ATP-eluted dnaK to CRAG. The ATP-eluted fraction was incubated with the phosphatase at 37°C for 15 min. Then 10 mM EDTA was added, and the sample was loaded onto the CRAG column again, washed, and eluted with ATP. After phosphatase treatment, 4-fold less of the loaded dnaK bound to the column (5% of the loaded material vs. 20% in the control). Shown are sequential 1-ml fractions eluted with ATP. Lanes: 1–4, without phosphatase; 5–8, after phosphatase treatment.

also alter other properties, such as dnaK's ATPase activity, its requirement for the cofactors dnaJ and grpE, the specificity of its binding to different peptides, or its tendency to form dimers. Most likely, phosphorylation results in some physiological advantage to the organism at high temperatures. Possibly, the phosphorylated dnaK is more efficient than the major form in catalyzing protein refolding (7), in preventing aggregation of unfolded polypeptides, or in facilitating the degradation of damaged proteins (12).

Although only a few percent of dnaK became phosphorylated upon heat shock, this adaptation increased 2- to 3-fold the amount of cellular dnaK capable of binding to CRAG or casein. In fact, only the modified form of dnaK appeared to bind to the unfolded protein column (Fig. 7). Moreover, with longer periods at 42°C, this fraction continued to increase, unlike the transcription of hsps, which rises and falls off within 15 min at 42°C (24). Thus, the time course of this modification and its biochemical consequences on chaperone function differ from the effect of increased transcription of this protein at 42°C. Presumably, both are important in adaptation to heat shock. The dnaK homolog in the endoplasmic reticulum, BiP, has also been reported to undergo phosphorylation *in vivo* and *in vitro* (25). This chaperone appears to associate with proteins as a monomer, and its phosphorylation was suggested to promote oligomer formation after protein dissociation (26).

Autophosphorylation of dnaK in the presence of high levels of Ca^{2+} has been reported by Zylicz *et al.* (17). Recently, McCarthy and Walker (18), M. Gottesman (Columbia University) (personal communication), and we (unpublished data) have found that incubation of pure dnaK at high temperatures stimulates its autophosphorylation. However, dnaK phosphorylated *in vitro*, unlike the form phosphorylated *in vivo*, described here, does not show enhanced binding to CRAG (unpublished data). Furthermore, autophosphorylation *in vitro* affects only Thr-199 in dnaK (18), but *in vivo*, both phosphothreonine and phosphoserine were reported in this protein (17). Thus, the physiological relevance of the autophosphorylation *in vitro* is uncertain, and an additional temperature-dependent kinase (or phosphatase) may function *in vivo* (see below). Interestingly, Gaut and Hendershot (30) found that BiP also undergoes autophosphorylation *in vitro* at the site homologous to the Thr-199 in dnaK, but *in vivo*, BiP is phosphorylated at different sites.

The modification of a fraction of the hsps leading to altered chaperone functions appears to be a general feature of the cell's response to high temperatures. At 42°C, groEL also changes its properties through phosphorylation (16); however, the resulting alterations in groEL's interactions with proteins differ from the phosphorylation-induced changes in dnaK's properties. Although the two species of groEL bind to the CRAG column similarly, the phosphorylated groEL dissociates from CRAG with ATP alone, whereas the unmodified form also requires groES for dissociation (unpublished data). In contrast, the phosphorylated dnaK dissociates normally from these ligands but shows much stronger binding to CRAG and casein than the predominant form. In fact, only the phosphorylated form bound to CRAG. Therefore, in cells growing at low temperatures, a fraction of dnaK and groEL must also be phosphorylated.

It is noteworthy that the time course of phosphorylation of these two chaperones at 42°C and their dephosphorylation upon shift back to 25°C were similar. Thus, these modifications appear to constitute coordinated cellular responses to temperature shifts. Possibly, a common kinase or phosphatase causes these modifications and functions in a temperature-dependent fashion. These responses to temperature shifts may be even more general, and it will be important to determine whether other chaperones are also modified reversibly under these conditions. In addition, it will be important to test whether the heat shock proteins homologous to dnaK (e.g., hsp70) in mitochondria and in the eukaryotic cytosol undergo similar modifications during heat shock.

After a mild heat shock, bacterial and eukaryotic cells become tolerant to high normally lethal temperatures (1). Although hsps are clearly essential for this protective effect, their levels do not correlate with resistance to heat killing (27, 28). For example, upon heat shock, yeast can acquire thermotolerance even when protein synthesis is blocked (27). Furthermore, upon down shift of many cells from 42°C to 30°C, the amounts of the hsps do not change, yet the cells rapidly lose their thermotolerance (28). As shown in Fig. 1, the heat-induced phosphorylation of groEL and dnaK increased with time at 42°C, while thermotolerance also increased progressively (data not shown). Moreover, these modifications were lost upon return to low temperatures when acquired thermotolerance was also lost, even though the total amounts of these chaperones were relatively unchanged. Therefore, we suggest that the heat-induced phosphorylation of dnaK and groEL may be critical in conferring the acquired thermotolerance, perhaps by enhancing the ability of the chaperones to repair or eliminate damaged cell proteins.

Hsp70 has been proposed to function as a "cellular thermometer" in the induction of the heat-shock response. Increased transcription of heat-shock genes at 42°C and in other stressful conditions is triggered by the accumulation in cells of damaged proteins (29). The heat-shock transcription factor σ^{32} is normally associated with dnaK, but during heat shock, denatured proteins may trap dnaK (19), which allows σ^{32} to stimulate transcription of heat-shock genes. Unfolded proteins may also signal the phosphorylation of dnaK. McCarthy and Walker (18) suggested that the heat-induced phosphorylation of dnaK may be part of the cell's temperature-sensing mechanism. Our findings suggest instead that the temperature-dependent phosphorylation of dnaK enhances its association with unfolded proteins. The resulting trapping of dnaK may allow σ^{32} to accumulate, which should stimulate expression of hsps. The phosphorylation or dephosphorylation of dnaK, by altering its association with proteins, thus may represent critical events in the biochemical transduction of changes in environmental temperature.

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1. Schlesinger, M., Ashburner, M. & Tissiers, R., eds. (1982) in *Heat Shock from Bacteria to Man* (Cold Spring Harbor Lab. Press, Plainview, NY).
2. Lindquist, S. & Craig, E. (1988) *Annu. Rev. Genet.* **22**, 631–677.
3. Neidhardt, F. C., van Bogelen, R. A. & Vaughn, U. (1989) *Annu. Rev. Genet.* **18**, 295–329.
4. Ellis, R. J. & van der Vies, S. (1991) *Annu. Rev. Biochem.* **60**, 321–347.
5. Goloubinoff, P., Gatenby, A. A. & Lorimer, G. H. (1989) *Nature (London)* **337**, 44–47.
6. Martin, J., Langer, T., Boteva, R., Schramel, A., Horwich, A. L. & Hartl, F.-U. (1991) *Nature (London)* **352**, 36–42.
7. Skowyra, D., Georgopoulos, C. & Zylicz, M. (1990) *Cell* **62**, 939–944.
8. Beckmann, R. P., Mizzen, L. E. & Welch, W. J. (1990) *Science* **248**, 850–854.
9. Paek, K.-H. & Walker, G. (1987) *J. Bacteriol.* **169**, 283–290.
10. Gaitanaris, G. A., Papavasiliou, A. G., Rubock, P., Silverstein, S. J. & Gottesman, M. E. (1990) *Cell* **61**, 1013–1020.
11. Liberek, K., Georgopoulos, C. & Zylicz, M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6632–6636.
12. Sherman, M. & Goldberg, A. L. (1992) *EMBO J.* **11**, 71–78.
13. Straus, D. B., Walter, W. A. & Gross, C. (1988) *Genes Dev.* **2**, 1851–1858.
14. Sherman, M. & Goldberg, A. L. (1991) *J. Bacteriol.* **173**, 4249–4256.
15. Hellebust, H., Uhlen, M. & Enfors, S.-O. (1989) *J. Biotechnol.* **12**, 275–284.
16. Sherman, M. & Goldberg, A. L. (1992) *Nature (London)* **357**, 167–169.
17. Zylicz, M., LeBowitz, J. H., McMacken, R. & Georgopoulos, C. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 6431–6435.
18. McCarthy, J. S. & Walker, G. C. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9513–9517.
19. Craig, E. & Gross, C. A. (1990) *Trends Biochem. Sci.* **16**, 135–138.
20. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
21. Phillips, T. A., Vaughn, V., Bloch, P. L. & Neidhardt, F. (1987) in *Escherichia coli and Salmonella typhimurium*, eds. Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M. & Umbarger, E. H. (Am. Soc. Microbiol., Washington, DC), pp. 919–966.
22. Manai, M. & Cozzone, A. J. (1979) *Biochem. Biophys. Res. Commun.* **91**, 819–826.
23. Schatz, P. J., Riggs, P. D., Jacq, A., Fath, M. J. & Beckwith, J. (1989) *Genes Dev.* **3**, 1035–1044.
24. Straus, D. B., Walter, W. A. & Gross, C. (1987) *Nature (London)* **329**, 348–351.
25. Welch, W. J., Garrels, J. I., Thomas, G. P., Lin, J. J.-C. & Feramisco, J. R. (1983) *J. Biol. Chem.* **258**, 7102–7111.
26. Freiden, P. J., Gaut, J. R. & Hendershot, L. M. (1992) *EMBO J.* **11**, 63–70.
27. Watson, K., Dunlop, G. & Caviciolli, R. (1984) *FEBS Lett.* **169**, 267–273.
28. Mackey, B. M. & Derrick, C. (1990) *J. Appl. Bacteriol.* **69**, 373–384.
29. Goff, S. A. & Goldberg, A. L. (1985) *Cell* **41**, 587–595.
30. Gaut, J. R. & Hendershot, L. M. (1993) *J. Biol. Chem.* **268**, 12691–12698.