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Monomerization of RepA dimers by heat shock proteins activates binding to DNA replication origin

(DnaJ/DnaK/plasmid P1)

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ABSTRACT **DnaK is a major heat shock protein of *Escherichia coli* and the homolog of hsp70 in eukaryotes. We demonstrate the mechanism by which DnaK and another heat shock protein, DnaJ, render the plasmid P1 initiator RepA 100-fold more active for binding to the P1 origin of replication. Activation is the conversion of RepA dimers into monomers in an ATP-dependent reaction and the monomer form binds with high affinity to *ori*P1 DNA. Reversible chemical denaturants also convert RepA dimers to monomers and simultaneously activate *ori*P1 DNA binding. Increasing protein concentration converts monomers to dimers and deactivates RepA. Based on our data and previous work, we present a model for heat shock protein action under normal and stress conditions.**

The synthesis of heat shock proteins (hsp) is increased in response to heat shock and other stress conditions. It is generally assumed that hsp protect against the effects of stress and restore the status quo; however, the mechanisms remain unclear (reviewed in ref. 1). Pelham (2) proposed a model for the mechanism of action of one of the most abundant and highly conserved families of hsp, hsp70. He proposed that heat stress partially denatures proteins, thereby exposing hydrophobic regions that coalesce into aggregates. hsp70 preferentially recognizes denatured proteins by binding to hydrophobic regions and inhibits, or even reverses, aggregation. A conformational change in hsp70, coupled to the hydrolysis of ATP, causes the denatured protein to be released. The released substrate then has a chance to refold before it aggregates. Skowyra *et al.* (3) have provided biochemical evidence for this model by finding that DnaK reactivates and also disaggregates heat-denatured RNA polymerase.

In nonstress conditions, DnaK protein comprises >1% of the total cell protein and plays an essential role in normal growth (4). It is involved in chromosome replication and segregation (5), as well as in the replication of phage λ and of plasmids mini-P1 and mini-F (reviewed in ref. 6). DnaK operates in conjunction with DnaJ and GrpE for the replication of λ, mini-P1, and mini-F. The function of the hsp in λ DNA replication has been studied extensively *in vitro* by Dodson *et al.* (7), Alfano and McMacken (8), and Zylicz *et al.* (9). Initiation requires the binding of the λO initiator protein to the DNA replication origin and the recruitment of the host DnaB helicase. This enzyme is brought to the origin by the simultaneous interaction of λP protein with both λO and DnaB. DnaK functions with DnaJ to release λP from its complex with DnaB and thus allow the DnaB helicase to unwind the DNA. Less DnaK is required if GrpE is also present (8, 9).

We have been studying the mechanism of action of the hsp in the initiation of plasmid mini-P1 replication. *In vitro* studies have shown that *ori*P1 replication catalyzed by crude protein fractions of *Escherichia coli* requires DnaK, DnaJ, and GrpE, along with many other host proteins (10, 11). This reaction also requires the P1 RepA initiator protein, which binds specifically to five 19-base-pair (bp) repeated sequences in the P1 origin (12). RepA, in addition to functioning in initiation, also regulates plasmid copy number by binding to nine sites in the control locus and regulates *repA* transcription by binding to sites in the origin that overlap the *repA* promoter (13).

RepA is a dimer in solution (11, 14) and forms a stable complex with DnaJ, containing a dimer each of RepA and DnaJ (11). DnaK, in a reaction dependent on DnaJ and ATP, activates the specific P1 origin DNA binding function of RepA (15). The activation reaction occurs prior to DNA binding. Following activation, only RepA is bound to the DNA. The electrophoretic mobility of the activated bound RepA was indistinguishable from that of the input 32-kDa RepA subunits. Thus, the change resulting in the activation of RepA is subtle.

We sought to define the change in RepA to understand the mechanism of action of DnaJ and DnaK. We show that DnaJ and DnaK activate RepA by converting RepA dimers to RepA monomers. From our results and the work of others, we present a general model for the action of hsp.

MATERIALS AND METHODS

Proteins and DNA. Proteins were purified as described (11). DNA fragments were prepared as described (15).

Assays for RepA Activation and for Activated RepA. To measure activation of RepA, reaction mixtures (20 μl) contained 20 mM Tris·HCl (pH 7.5), 40 mM KCl, 100 mM NaCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 50 μM ATP, bovine serum albumin (50 μg/ml), calf thymus DNA (50 μg/ml), 50 fmol of labeled *ori*P1 DNA fragment, 30 ng of DnaJ, 100 ng of DnaK, and RepA. Incubations were for 20 min at 24°C. Reaction mixtures were then diluted with 50 μl of wash buffer (20 mM Tris·HCl, pH 7.5/0.1 mM EDTA/1 mM dithiothreitol/10 mM $MgCl_2$/40 mM KCl/100 mM NaCl) and immediately filtered with gentle suction through nitrocellulose filters (25-mm Millipore type HA; 0.45 μm) that had been soaked in wash buffer. Filters were washed once with 1 ml of buffer, dried, and radioactivity was measured in a liquid scintillation counter. For gel-retardation experiments, 2 μl of a solution of 20% (wt/vol) sucrose, 0.1% (wt/vol) bromphenol blue, and 0.1 mM EDTA was added to each reaction mixture. Samples (11 μl) were applied to 5% acrylamide gels containing TBE buffer (89 mM Tris borate, pH 8.8). Electrophoresis was for 2.5 hr at 10 V/cm in TBE buffer. The gels were fixed in 10% (vol/vol) acetic acid containing 10% (vol/vol) methanol, transferred to Whatman 3MM paper, dried, and autoradiographed.

To measure activated RepA, reaction mixtures were assembled as described above, but with the omission of ATP,

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Abbreviation: hsp, heat shock protein(s).

DnaJ, and DnaK. Incubations were carried out at 0°C for 5 min and then processed as described above.

RESULTS

Isolation of Activated RepA. Since RepA *ori*P1 DNA binding activity is stimulated 100-fold in an ATP-dependent reaction catalyzed by DnaJ and DnaK (15), we wanted to determine whether the RepA that was bound to the *ori*P1 DNA could be released in the activated form. RepA–DNA complexes were isolated by gel filtration after incubation of RepA with DnaJ, DnaK, ATP, and ^{3}H-labeled *ori*P1 DNA (Fig. 1*A*). About half of the DNA was bound to RepA and the purification yield was ≈90%. The RepA–DNA complex was then dissociated by treating with NaCl (Table 1). In the presence of NaCl, RepA was no longer bound to the DNA. However, when the concentration of NaCl was lowered by dilution, RepA once again bound to the ^{3}H-labeled *ori*P1 DNA at 0°C. DNA binding of RepA was not stimulated by incubation with DnaJ, DnaK, and ATP at 24°C. We previously showed that the activated form of RepA can be distinguished from the unactivated form by its ability to bind to *ori*P1 DNA in <30 sec at 0°C; the activation of RepA by DnaJ, DnaK, and ATP does not occur at 0°C but requires incubation at 24°C for 20 min (15). These results suggested that the RepA protein could be dissociated at high ionic strength from DNA in the active form.

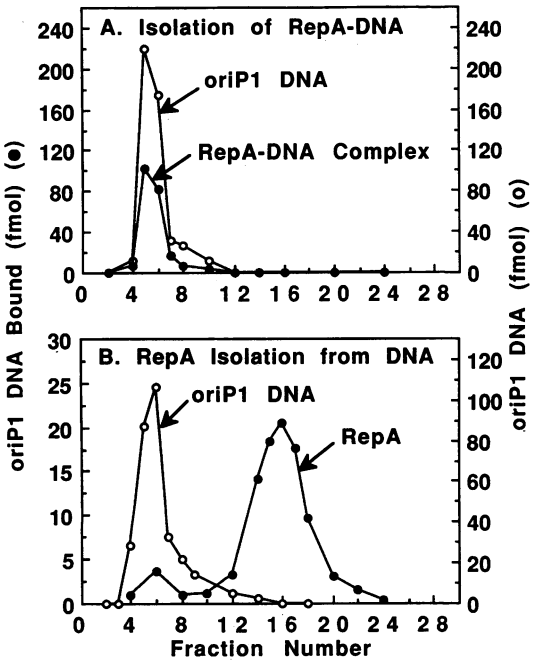

FIG. 1. Isolation of the activated form of RepA. (*A*) A 1-ml reaction mixture as described in *Materials and Methods* for the activation of RepA containing 2 μg of RepA, 2 μg of DnaJ, 25 μg of DnaK, and 6.3 pmol of ^{3}H-labeled *ori*P1 DNA (1182-bp DNA fragment) was incubated for 20 min and applied to a Sepharose 6B column (1 × 27 cm) equilibrated with buffer A [5% (vol/vol) glycerol/20 mM Tris·HCl, pH 7.5/0.1 mM EDTA/1 mM dithiothreitol] containing 20 mM KCl and 100 mM NaCl. The column was eluted with the same buffer and the position of the eluted DNA was determined by counting a portion of each fraction (○). A portion of each fraction was also collected on a nitrocellulose filter to determine the amount of protein-bound DNA (●). (*B*) The RepA–*ori*P1 DNA complex (fraction 5; 500 μl) was adjusted to 0.5 M NaCl and 100 μg of bovine serum albumin per ml, incubated at 0°C for 30 min, and applied to a Sepharose 6B column equilibrated with buffer A containing 0.4 M NaCl and 100 μg of bovine serum albumin per ml. Position of the DNA was determined by counting a portion of the fractions (○). A portion of each fraction was assayed for *ori*P1 DNA binding activity at 0°C (●).

Table 1. Dissociation of RepA from DNA in the active form

	*ori*P1 DNA bound, fmol
RepA–^{3}H-labeled *ori*P1 DNA complex	18
Complex treated with NaCl	1
NaCl treated, diluted	16
NaCl treated, diluted, incubated with DnaJ, DnaK, and ATP	14

RepA–DNA complex was purified as described in Fig. 1*A*. The complex was treated with 0.75 M NaCl for 10 min at 0°C, where indicated. The mixtures were diluted 1:5 with buffer A (Fig. 1*A*). Each reaction mixture contained 42 fmol of ^{3}H-labeled *ori*P1 DNA from the original RepA–DNA complex. DNA binding was measured by retention on nitrocellulose filters. Incubations in the absence of DnaJ and DnaK were at 0°C; that in the presence of DnaJ and DnaK was at 24°C.

We showed directly that RepA was released from DNA in the activated state by purifying RepA away from *ori*P1 DNA by gel filtration chromatography after dissociation of the protein–DNA complex with 1 M NaCl (Fig. 1*B*). All of the *ori*P1 DNA binding activity was now included in the column and well separated from the free DNA and protein–DNA complex. About 80–90% of the activity applied to the column was recovered. We analyzed the activity of the released RepA by gel retardation assays (Fig. 2). RepA released from DNA was in the active form because (*i*) it bound to *ori*P1 DNA at 0°C (Fig. 1*B*); (*ii*) DNA binding was not further stimulated by incubation with DnaJ, DnaK, and ATP (Fig. 2); and (*iii*) its specific activity was similar to that of the activated form of RepA (Fig. 2) and 50- to 100-fold higher than that of the unactivated form. If RepA had been released in the inactive form, we would have expected ≈1% recovery of DNA binding activity and DNA binding would have been further activated by DnaJ, DnaK, and ATP. Thus, the activated form of RepA is quite stable after its separation from DnaJ, DnaK, ATP, and DNA.

The Activated Form of RepA Is a Monomer. DnaK and hsp70 have been implicated in the disaggregation of proteins, suggesting that DnaK might carry out that function in activating RepA. However, purified RepA does not appear to aggregate under our reaction conditions (15). By analogy with the disaggregation role of DnaK, it seemed possible that DnaK and DnaJ were catalyzing the dissociation of RepA dimers into monomers and that this was the form needed for origin binding. This possibility was also attractive because the 19-bp RepA binding site has no obvious twofold symmetry, characteristic of dimeric prokaryotic DNA binding proteins.

To test this possibility, we analyzed the activated form of RepA by gel filtration under conditions that separate RepA monomers and dimers. Purified RepA chromatographed as a protein of 70 kDa (Fig. 3*A*) and therefore as the dimer of 32-kDa promoters. The activated form of RepA chromatographed as a protein of 27 kDa (Fig. 3 *B* and *C*). This result in conjunction with the previous result that the activated form of RepA was indistinguishable from the unactivated form by

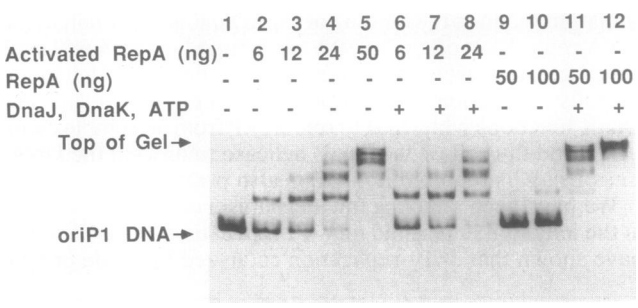

FIG. 2. Analysis of the activated form of RepA by gel retardation. Reactions were performed as described in *Materials and Methods*.

SDS/PAGE analysis (15) indicates that the activated form of RepA is a monomer. This experiment did not rule out the possibility that there was an additional conformational change or modification in RepA that was involved in converting it to the activated form.

Chemical Dissociation of RepA Activates RepA. The observation that DnaK, DnaJ, and ATP convert RepA dimers into monomers suggested that it might be possible to chemically dissociate RepA dimers to produce activated RepA. RepA protein was treated with NaCl, urea, guanidine hydrochloride, Triton X-100, or SDS. The samples were dialyzed to remove the salt or denaturant and then assayed alone for *ori*P1 DNA binding and with DnaJ, DnaK, and ATP (Table 2). Treatment with 0.4 or 5 M NaCl did not activate RepA. However, the protein was still in a native state as measured by its activation by DnaJ, DnaK, and ATP. In contrast, treatment with urea or guanidine resulted in conversion of RepA to the activated form. DnaJ, DnaK, and ATP stimulated binding by ≈2-fold, most likely due to activation of RepA dimers remaining or reforming after the treatment. Activation by urea depended on having a low protein concentration during the treatment. With RepA at 2–5 μg/ml, *ori*P1 DNA binding was the same with or without DnaJ, DnaK, and ATP; at 10–20 μg/ml, binding without DnaJ, DnaK, and ATP was 2- to 4-fold lower; and at 100 μg/ml, binding without DnaJ, DnaK, and ATP was 10- to 20-fold lower. Treatment with Triton X-100 partially activated RepA. Treatment with SDS also partially activated RepA; however, the stimulation by DnaJ, DnaK, and ATP was less, suggesting that some RepA had irreversibly denatured.

Table 2. Activation of RepA by chemical denaturation

	*ori*P1 DNA bound, fmol	
	Without DnaJ, DnaK, ATP	With DnaJ, DnaK, ATP
Untreated RepA	<1	27
0.4 M NaCl treated	1	37
5 M NaCl treated	1	30
8 M urea treated	26	42
4 M guanidine treated	18	30
0.5% Triton X-100 treated	9	40
0.1% SDS treated	7	14

RepA was diluted to 10 μg/ml in buffer A (see Fig. 1*A*) containing 50 μg of bovine serum albumin per ml and 50 mM NaCl, 400 mM NaCl, 5 M NaCl, 8 M urea, 4 M guanidine hydrochloride, 0.5% Triton X-100, or 0.1% SDS. After 30 min at 0°C, samples were dialyzed against 200 ml of buffer A plus 400 mM NaCl. Twenty nanograms of each sample was assayed for DNA binding activity with or without DnaJ, DnaK, and ATP by nitrocellulose filter binding.

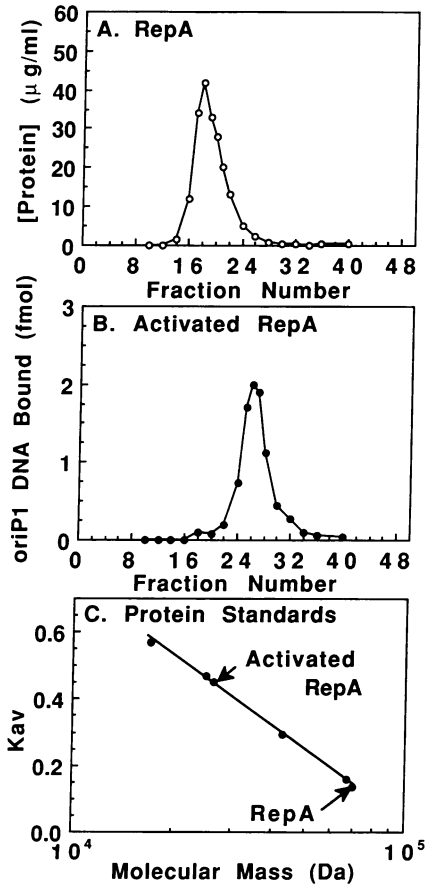

FIG. 3. G100 column chromatography of RepA and the activated form of RepA. (*A*) Purified RepA (0.2 ml, 100 μg) was applied to a G100 column (1 × 27 cm) equilibrated with buffer A containing 0.4 M NaCl. The column was eluted with the same buffer, fractions were collected, and protein was measured. (*B*) Activated RepA (0.2 ml) released from DNA (described in Fig. 1) was applied to the G100 column as in *A* but with 50 μg of bovine serum albumin per ml in the column buffer. Fractions were collected and assayed for *ori*P1 DNA binding at 0°C minus DnaJ, DnaK, and ATP. (*C*) Protein standards, bovine serum albumin, ovalbumin, chymotrypsinogen, and myoglobin (Pharmacia) were applied separately to the G100 column and protein was measured. K_{av} values were calculated for RepA and the protein standards and were plotted as a function of their known molecular masses. The K_{av} value for the activated form of RepA was also plotted.

Urea-treated RepA (at low protein concentration) chromatographed as a monomer on G100 gel filtration, as assayed by *ori*P1 DNA binding at 0°C (Fig. 4*A*). A very small peak of dimeric RepA was detected, as assayed by the stimulation of binding by DnaJ, DnaK, and ATP. Untreated RepA, at the same protein concentration, migrated as a dimer (Fig. 4*B*). The urea-activated RepA and the protein-activated RepA had similar specific activities for DNA binding as measured by

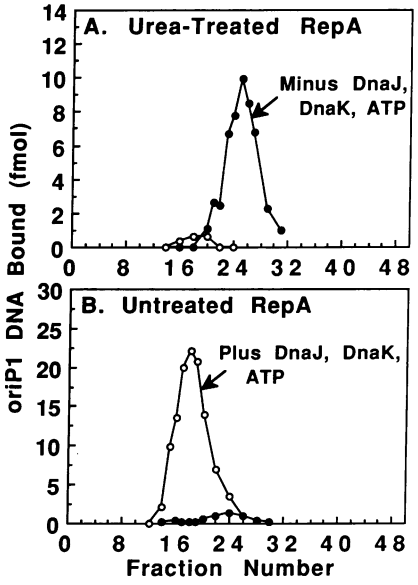

FIG. 4. G100 column chromatography of urea-treated RepA. (*A*) RepA (5 μg, 200 μl) was adjusted to 8 M urea and 0.4 M NaCl in buffer A and incubated 30 min at 0°C. The sample was dialyzed and applied to a G100 column as described in Fig. 3. (*B*) RepA (5 μg, 200 μl) was adjusted to 0.4 M NaCl in buffer A. The sample was chromatographed as described above. Fractions from both columns were assayed for *ori*P1 DNA binding at 0°C without DnaJ, DnaK, and ATP (●) and at 24°C with DnaJ, DnaK, and ATP (○).

nitrocellulose filter binding and gel retardation (data not shown). These experiments show that the activation of RepA catalyzed by DnaJ, DnaK, and ATP can be mimicked by chemically converting RepA dimers to monomers. They suggest that DnaJ and DnaK do not modify RepA but simply dissociate it into monomers, which bind to *ori*P1 DNA with high affinity. Thus, it is sufficient for DnaK to catalyze only the dissociation of RepA dimers. If DnaK simultaneously unfolds RepA monomers, the monomers can refold without DnaJ and DnaK.

Conversion of Monomers to Dimers Reverses Activation of RepA. The observation that urea converts RepA dimers to stable monomers only when the protein concentration is low suggested that dimers could be re-formed by increasing the protein concentration. RepA, at a concentration of 10 μg/ml, was treated with urea, dialyzed, and then concentrated to 100 μg/ml. Before concentration, urea-treated RepA was largely in the activated form; RepA bound to DNA at 0°C and DNA binding was stimulated only 2-fold by incubation with DnaJ, DnaK, and ATP (Fig. 5*A*). After concentration, RepA was in the unactivated form; DNA binding by RepA was 10-fold lower. However, DnaJ, DnaK, and ATP stimulated the binding activity ≈10-fold and restored the activity almost to the original level of the urea-treated RepA (Fig. 5*B*). Similar experiments with RepA monomers generated by the action of DnaJ and DnaK have not been performed. However, this reversibility of the monomer and dimer forms of RepA confirms that activation and deactivation need not involve a covalent change in RepA, such as phosphorylation or dephosphorylation, catalyzed by DnaJ and DnaK. Moreover, the failure of the dimers to dissociate in the absence of hsp or denaturants may be the result of a kinetic block.

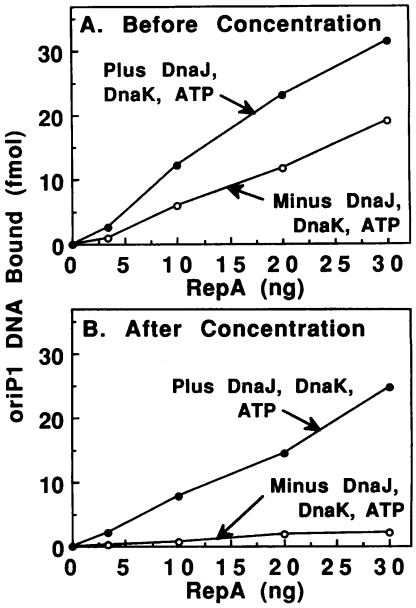

FIG. 5. Reversal of activation by protein concentration. RepA was treated with 8 M urea and dialyzed as described in Table 2. A Centricon-10 microconcentrator (Amicon) was then used to concentrate the sample 10-fold. *ori*P1 DNA binding was measured before (*A*) and after (*B*) concentration, both without DnaJ, DnaK, and ATP at 0°C (○) and with DnaJ, DnaK, and ATP at 24°C (●).

DISCUSSION

Our results suggest the following mechanism of activation of RepA. RepA dimers bind rapidly with DnaJ dimers to form

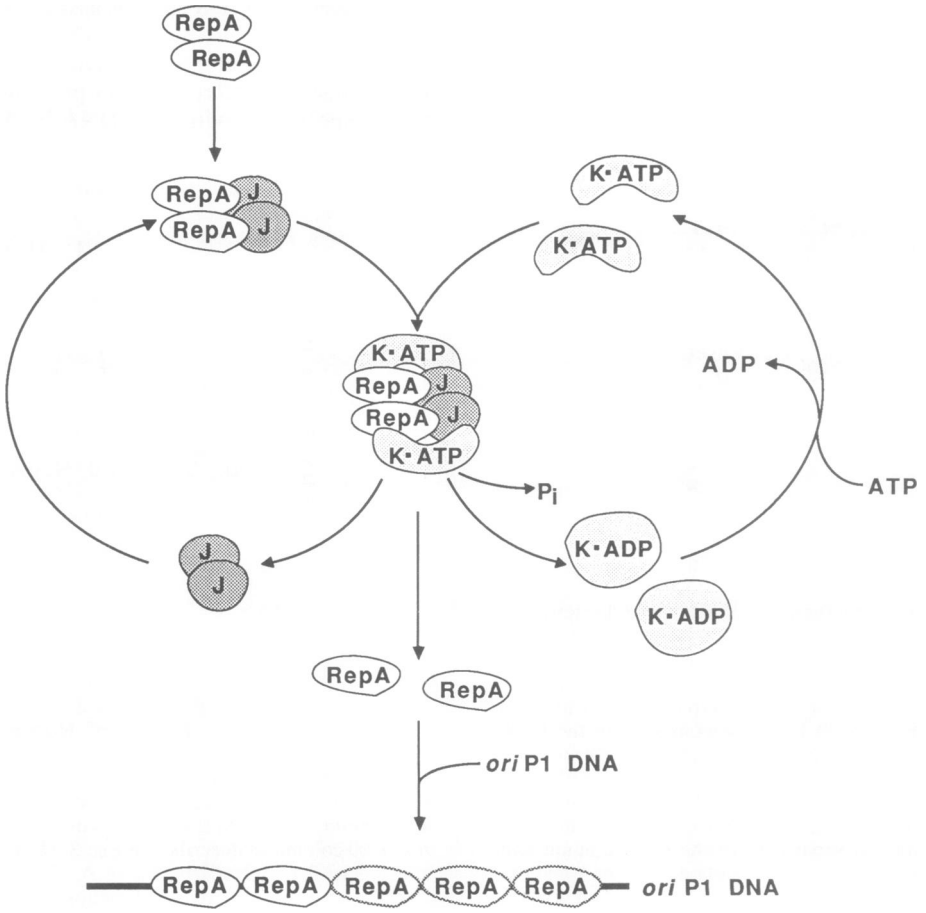

FIG. 6. Model of the mechanism of action of DnaJ and DnaK on native proteins. See *Discussion*.

$RepA_2$–$DnaJ_2$ complexes (11). DnaJ functions to target RepA for recognition by DnaK. DnaK then dissociates the $RepA_2$–$DnaJ_2$ tetramer releasing 2 $RepA_1$ and $DnaJ_2$ in a slower ATP-dependent reaction. Once converted to monomers, $RepA_1$ is stable at dilute protein concentration and can bind to DNA rapidly without further involvement of DnaK, DnaJ, or ATP. Urea treatment at dilute protein concentration followed by dialysis also converts RepA dimers to monomers and activates the DNA binding function of RepA. Increasing the protein concentration of RepA monomers reverses the activation. RepA monomers may be active for DNA binding because the DNA binding domain overlaps the dimerization domain and that domain becomes exposed upon monomerization. Thus, DnaJ and DnaK regulate protein function at the level of protein structure.

DnaJ and DnaK also activate DNA binding by RepA *in vivo*. Mini-P1 plasmids can transform *dnaK* mutant strains and *dnaJ* and *grpE* deletion strains but are unstably maintained (5, 16). Moreover, Tilly *et al.* (17) have shown that repression of the autoregulated *repA* promoter by RepA is 2- to 10-fold less in *dnaJ*, *dnaK*, and *grpE* mutant strains compared to wild-type strains. These results can be explained by our finding that DnaJ and DnaK activate the DNA binding function of RepA by converting dimers to monomers. GrpE is not essential for activation of RepA *in vitro*. As suggested for λ replication, GrpE may function in recycling DnaK (6). The *in vivo* results suggest that RepA exists as an inactive dimer and that it is the monomer form of RepA that is active in DNA replication. We have observed that RepA monomers are active in *in vitro ori*P1 DNA replication reactions (unpublished data). It is not clear how RepA dimers arise *in vivo* and what their role is. It is likely that the dimer form is involved in regulating P1 DNA replication. Dimerization may be a mechanism for sequestering RepA and thus limiting DNA replication, since we have shown that RepA dimers bind poorly to P1 DNA (15).

A more general model of how DnaJ and DnaK might function catalytically is shown in Fig. 6. It is analogous to that proposed by Pelham for the heat shock response, but differs in that it emphasizes the role of hsp with native proteins in unstressed conditions. Clearly, hsp would pose a danger to the cell if they denatured or dissociated active proteins or complexes. In the heat shock response, it is thought that damaged proteins are identified as targets for DnaK through the specific recognition of exposed hydrophobic regions (2). In normal growth conditions, native proteins are identified as targets by DnaK through the specific recognition of a protein tag. In our case, DnaJ tags RepA for binding by DnaK, perhaps also by exposing hydrophobic regions. In the case of λ DNA replication, it is likely that DnaJ tags λP for binding by DnaK. DnaK then catalyzes the dissociation of λP from DnaB in the intiation complex.

By analogy with other processes involving ATP-dependent conformational coupling, there may be two conformations of DnaK. The stable form in the absence of ATP does not bind to the target protein, whereas the ATP-bound form does. When DnaK binds to the RepA–DnaJ complex, either RepA or DnaJ acts as an allosteric effector of the DnaK ATPase. The hydrolysis of ATP leads to the return of DnaK to the ground-state conformation and to its release from the complex. The binding of DnaK to RepA leads to dissociation of RepA dimers and thereby DnaJ is also released because only the dimer form of RepA binds DnaJ. Thus, DnaK bound to ATP acts like urea, which is known to bind to hydrophobic regions and thereby promote denaturation and depolymerization (18). ATP hydrolysis and the accompanying conformational change of DnaK is then the analogue of the removal of urea by dialysis. This step allows spontaneous refolding to the active form of the target.

It is anticipated that by studying the relatively simple three-component system that we have described here, more details of the mechanism of function of hsp will become clear.

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1. Morimoto, R. I., Tissieres, A. & Georgopoulos, C. (1990) in *Stress Proteins in Biology and Medicine*, eds. Morimoto, R., Tissieres, A. & Georgopoulos, C. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 1–36.
2. Pelham, H. B. R. (1986) *Cell* **46**, 959–961.
3. Skowyra, D., Georgopoulos, C. & Zylicz, M. (1990) *Cell* **62**, 939–944.
4. Bukau, B. & Walker, G. (1989) *J. Bacteriol.* **171**, 2337–2346.
5. Bukau, B. & Walker, G. (1989) *J. Bacteriol.* **171**, 6030–6038.
6. Georgopoulos, C., Ang, D., Liberek, K. & Zylicz, M. (1990) in *Stress Proteins in Biology and Medicine*, eds. Morimoto, R., Tissieres, A. & Georgopoulos, C. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 191–222.
7. Dodson, M., Echols, H., Wickner, S., Alfano, C., Mensa-Wilmot, K., Gomes, B., LeBowitz, J., Roberts, J. & McMacken, R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 7638–7642.
8. Alfano, C. & McMacken, R. (1989) *J. Biol. Chem.* **264**, 10709–10718.
9. Zylicz, M., Ang, D., Liberek, K. & Georgopoulos, C. (1989) *EMBO J.* **8**, 1601–1608.
10. Wickner, S. & Chattoraj, D. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 3668–3672.
11. Wickner, S. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 2690–2694.
12. Abeles, A. L., Snyder, K. M. & Chattoraj, D. K. (1984) *J. Mol. Biol.* **173**, 307–324.
13. Chattoraj, D. K., Snyder, K. M. & Abeles, A. L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2588–2592.
14. Swack, J. A., Pal, S. K., Mason, R. J., Abeles, A. L. & Chattoraj, D. K. (1987) *J. Bacteriol.* **169**, 3737–3742.
15. Wickner, S., Hoskins, J. & McKenney, K. (1991) *Nature (London)* **350**, 165–167.
16. Tilly, K. & Yarmolinsky, M. (1989) *J. Bacteriol.* **171**, 6025–6029.
17. Tilly, K., Sozhamannan, S. & Yarmolinsky, M. (1990) *New Biol.* **2**, 1–6.
18. Tanford, C. (1970) *Adv. Protein Chem.* **24**, 1–95.