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The Escherichia coli Chaperones Involved in DNA Replication [and Discussion]

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Phil. Trans. R. Soc. Lond. B 1993 **339**, 271-278
doi: 10.1098/rstb.1993.0025

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The *Escherichia coli* chaperones involved in DNA replication

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SUMMARY

Mutations in the *Escherichia coli* heat shock genes, *dnaK*, *dnaJ* or *grpE*, alter host DNA and RNA synthesis, degradation of other proteins, cell division and expression of other heat shock genes. They also block the initiation of DNA replication of bacteriophages λ and P1, and the mini-F plasmid. An *in vitro* λDNA replication system, composed entirely of purified components, enabled us to describe the molecular mechanism of the *dnaK*, *dnaJ* and *grpE* gene products. DnaK, the bacterial hsp 70 homologue, releases λP protein from the preprimosomal complex in an ATP- and DnaJ-dependent reaction (GrpE-independent initiation of λDNA replication). In this paper, I show that, when GrpE is present, λP protein is not released from the preprimosomal complex, rather it is translocated within the complex in such a way that it does not inhibit DnaB helicase activity. Translocation of λP triggers the initiation event allowing DnaB helicase to unwind DNA near the *ori*λ sequence, leading to efficient λDNA replication. Chaperone activity of the DnaK–DnaJ–GrpE system is first manifested in the selective binding of these heat shock proteins to the preprimosomal complex, followed by its ATP-dependent rearrangement. I show that DnaJ not only tags the preprimosomal complex for recognition by DnaK, but also stabilizes the multi-protein structure. GrpE also participates in the binding of DnaK to the preprimosomal complex by increasing DnaK's affinity to those λP proteins which are already associated with DnaJ. After attracting DnaK to the preprimosomal complex, DnaJ and GrpE stimulate the ATPase activity of DnaK, triggering conformational changes in DnaK which are responsible for the rearrangement of proteins in the preprimosomal complex and recycling of these heat shock proteins. The role of DnaK, DnaJ and GrpE in λDNA replication is in sharp contrast to our understanding of their role in the *oriC*, P1, and probably mini-F DNA replication systems. In the cases of *oriC* and P1 DNA replication, these heat shock proteins activate initiation factors before they are in contact with DNA, and are not required during the subsequent steps leading to the initiation of DNA replication. The common feature of DnaK, DnaJ and GrpE action in these systems is their ATP-dependent disaggregation or rearrangement of protein complexes formed before or during initiation of DNA replication.

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1. INTRODUCTION

In 1971, Georgopoulos & Herskowitz isolated *Escherichia coli* mutations in the *dnaK*, *dnaJ* and *grpE* genes which do not allow initiation of bacteriophage λDNA replication. Mutations in the *dnaK*, *dnaJ* or *grpE* genes have also been shown to confer similar phenotypes with respect to bacterial physiology and gene regulation (for review, see Gross *et al.* 1990; Georgopoulos *et al.* 1990; Ang *et al.* 1991). These include: (i) the overproduction of all heat shock proteins, even at non-heat shock temperatures; (ii) a block on host DNA and RNA synthesis at non-permissive temperatures; and (iii) a defect in proteolysis.

These genes have subsequently been shown to encode heat shock proteins (Georgopoulos *et al.* 1990). The amino acid sequences of these proteins have been conserved throughout evolution (Craig & Gross 1991). Among the eukaryotic and prokaryotic DnaK (hsp 70) homologues, for example, this conservation is approximately 50% identical at the amino acid level (Bardwell & Craig 1984). Protein structure and amino acid sequence analysis have led to the realization that the functionally diverse actin, hexokinase, and hsp 70 protein families have a common ATPase domain of similar three-dimensional structure (Bork *et al.* 1992).

Recent work suggests that hsp 70s play a central role in cell viability under normal and stress conditions. This work involves binding to and release from other polypeptides to facilitate (or prevent) inter- and intramolecular interactions (reviewed in Craig & Gross 1991). The combined efforts of several laboratories led to the realization that these proteins are chaperones which are involved in protein folding and refolding, DNA and RNA synthesis, proteolysis, protein transport through membranes, disaggregation and protection of other enzymes from heat inactivation (reviewed in Rothman 1989; Ang *et al.* 1991; Craig & Gross 1991; Langer *et al.* 1992; Georgopoulos 1992). The hsp 70 proteins have several biochemical properties in common: (i) they all have an extremely

Phil. Trans. R. Soc. Lond. B (1993) **339**, 271–278
Printed in Great Britain

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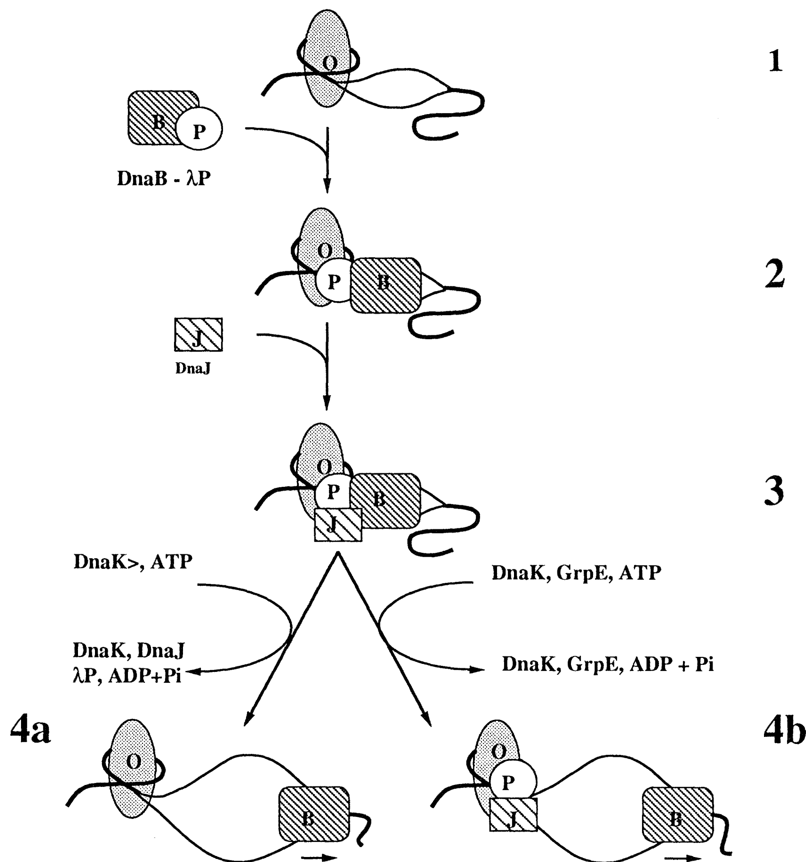

Figure 1. Hypothetical model of the steps involved in initiation of λDNA replication (see text for details). λO protein binds to *ori*λ sequences (step 1) and attracts DnaB helicase, modified by λP, to the *ori*λ sequence (step 2). Heat shock protein DnaJ stabilizes the preprimosomal complex and tags the preprimosomal complex for recognition by DnaK (step 3). When a high amount of DnaK protein is used (step 4*a*), in an ATP-dependent reaction, DnaK and DnaJ release λP protein from the complex, allowing DnaB helicase to unwind λDNA and leading to DNA synthesis. In the presence of GrpE (step 4*b*), 10 times less DnaK protein is required for efficient λDNA replication. In this case, λP is not released from the preprimosomal complex but unwinding still occurs. In this paper, I propose that in the GrpE-dependent reaction (when the lower concentration of DnaK protein is used), λP protein is translocated into the preprimosomal complex in such a way that it is no longer in contact with DnaB helicase.

weak ATPase activity (Zylicz *et al.* 1983); (ii) they display promiscuous binding to many native and unfolded proteins (Rothman 1989), including interactions with many newly synthesized proteins (Beckmann *et al.* 1990); (iii) for all investigated hsp 70 proteins, there is a perfect correlation between ATP hydrolysis, the release of bound polypeptides, and dramatic conformational changes (Liberek *et al.* 1991*a*; Palleros *et al.* 1992; Banecki *et al.* 1992); (iv) some of them have been shown to be able to participate in the protection of enzymes from thermal inactivation (Pelham 1988; Skowyra *et al.* 1990); and (v) an ability to disaggregate protein aggregates has also been demonstrated for these proteins (Pelham 1988). In the case of DnaK, these aggregates include the preprimosomal assembly at *ori*λ (Liberek *et al.* 1988; Dodson *et al.* 1989; Alfano & McMacken 1989*b*), P1 RepA dimers (Wickner *et al.* 1991*a*,*b*), aggregates of DnaA (Hwang *et al.* 1990) and aggregates of heat-inactivated RNA polymerase (Skowyra *et al.* 1990).

The *dnaK*, *dnaJ* and *grpE* gene products have all been successfully purified to homogeneity by using an *in vitro* λ DNA replication complementation assay (Zylicz & Georgopoulos 1984; Zylicz *et al.* 1985, 1987). This has allowed analysis of their individual and combined activities in well-defined reconstituted λDNA replication systems (Mensa-Wilmot *et al.* 1989; Zylicz *et al.* 1989).

2. λDNA REPLICATION

In vitro reconstitution of a λDNA replication system, using highly purified bacteriophage λ and *E. coli* replication proteins, enabled the identification and description of several replication intermediates (figure 1).

1. The bacteriophage-encoded λO replication protein specifically recognizes the *ori*λ DNA sequence located within the λ*O* gene sequence. The λO dimers not only bind to four repeated sequences, but also form the large 'λO-some' structure (Dodson *et al.* 1985). In the presence of supercoiled λDNA, binding of λO protein to the *ori*λ sequences causes destabilization of the duplex DNA near an AT-rich region of *ori*λ sequence, resulting in the formation of the 'open complex' (Schnos *et al.* 1988).

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Table 1. *Isolation of λDNA replication intermediates*

(The 120 μl reaction containing the prepriming proteins listed (including ^{14}C-labelled λP protein and λdv plasmid DNA) was pre-incubated for 12 min at 32°C, using concentrations and buffer conditions described by Zylicz *et al.* (1989), and applied to a Sepharose 4B column as described by Liberek *et al.* (1988). The 100 μl fractions collected from the column and 17 μl of the void volume fraction (fraction 4), containing λDNA and protein associated with it, were assayed in the purified, reconstituted replication system as described by Zylicz *et al.* (1989). Missing prepriming proteins, if any, along with DnaG primase and DNA polymerase III holoenzyme, were added at this time, and incorporation of ^{3}H-TTP to DNA was determined as described by Zylicz *et al.* (1989). Before applying the 120 μl reaction to the column, a 17 μl portion was removed and incubated at room temperature (during the time when the rest of the volume was chromatographed), and assayed in the purified replication system. This control was used to estimate the recovery of DNA replication-competent λDNA. After chromatography on Sepharose 4B, 50 μl of each fraction was used to determine the amount of ^{14}C-labelled λP protein present in the complex with λDNA as described by Liberek *et al.* (1988).)

λDNA replication intermediates	percentage recovery of λDNA replication activity	percentage of λP in complex with λDNA
1. ori λ–λO	80	—
2. ori λ–λO–λP–DnaB	70	63
3. ori λ–(λO–λP–DnaB) DnaJ	60	80
4*a*. ori λ–(λO–λP–DnaB) DnaJ, DnaK > > >	20	3
4*b*. ori λ–(λO–λP–DnaB) DnaJ, DnaK, GrpE	70	60

2. The other bacteriophage λ-encoded replication protein, λP, is thought to function in bringing DnaB helicase to the *ori*λ sequences. Because the λP protein binds more tightly to DnaB than DnaC does to DnaB, the λP protein can efficiently 'sequester' DnaB helicase for the specific initiation of λDNA replication (Wickner 1978; Alfano & McMacken 1988). The λP-DnaB complex is localized to the *ori*λ site through specific protein–protein interactions between λP and λO (Zylicz *et al.* 1984). The stable *ori*λ–λO–λP–DnaB complex can be assembled on supercoiled, relaxed or linear λDNA (Alfano & McMacken 1988), but it is still not clear if the structure and stoichiometry of these complexes are the same. The three-dimensional structure of the *ori*λ–λO–λP–DnaB complex assembled on supercoiled λDNA is also unknown. In particular, it is not clear if DnaB helicase is already positioned at the junction of double-stranded and single-stranded DNA created in the AT-rich region of *ori*λ sequence during binding of λO protein. However, the resulting *ori*λ–λO–λP–DnaB complex is stable, as it can be purified by gel filtration and subsequently demonstrated to be still active in λDNA replication in the absence of further λO, λP, or DnaB additions (table 1; see also Dodson *et al.* 1985; Liberek *et al.* 1988; Alfano & McMacken 1989*a*; Zylicz *et al.* 1989). The strong λP–DnaB interaction that assists in the localization of DnaB helicase to the *ori*λ sequences now inhibits the helicase activity of the DnaB protein (Wickner 1978; LeBowitz & McMacken 1986). Subsequent reactions are therefore needed to liberate DnaB helicase from the complex with λP.

3. The *E. coli dnaJ* gene product binds to the *ori*λ–λO–λP–DnaB intermediate by means of interactions with λP, λO, and DnaB proteins (Alfano & McMacken 1989*a*; Zylicz *et al.* 1989; Liberek *et al.* 1991*b*; Osipiuk *et al.* 1993). Although the presence of DnaJ stabilizes the preprimosomal complex (table 1; also M. Zylicz, unpublished results), primarily by stabilizing λO–λP interactions (figure 2), it has also been shown (Liberek *et al.* 1988) that there is an absolute requirement for the presence of DnaJ for DnaK to release λP (see below).

4. In the absence of GrpE, DnaK binds to λP. Subsequent ATP hydrolysis by DnaK leads to the release of λP from the complex (Liberek *et al.* 1990). The binding of DnaK to the preprimosomal structure is apparently transient because it has not been possible to isolate a DNA replication intermediate in which DnaK is found in a complex with *ori*λ–(λO–λP–DnaB)–DnaJ. Recently, we have been able to show that dimers and higher order multimeric forms of DnaK, which formed when the DnaK protein concentration is increased, have at least a seven-times higher affinity for λP than do monomers of DnaK (Osipiuk *et al.* 1993). These experiments help to explain our earlier observations that, in the absence of GrpE, high concentrations of DnaK are required for the initiation of λDNA replication (Zylicz *et al.* 1989). The significance of these multimeric forms of DnaK was recently suggested by genetic experiments (Wild *et al.* 1992). The net effect of the addition of DnaK and ATP to the *ori*λ–(λO–λP–DnaB)–DnaJ preprimosomal complex is the release of λP from the complex, allowing DnaB helicase to start unwinding the DNA at the *ori*λ sequence (table 1; see also Dodson *et al.*, 1986, 1989; Liberek *et al.* 1988; Alfano & McMacken 1989*b*). This unwinding proceeds in one direction, leading to unidirectional replication (Mensa-Wilmot 1989; Dodson *et al.* 1989). The DnaK- and DnaJ-dependent release of λP is not very specific, in that only about one fifth of the λDNA molecules are observed to unwind as a consequence of λP release. For the remaining molecules, addition of DnaK leads to the release of not only λP, but also DnaB, and even some λO protein from the complex with λDNA, and for these DNA molecules no replication occurs (table 1).

5. The presence of GrpE allows a tenfold reduction

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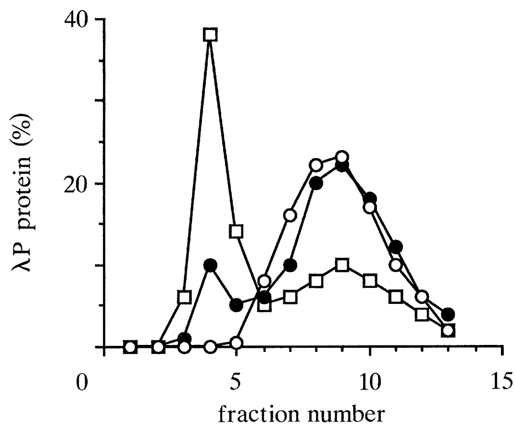

Figure 2. DnaJ heat shock protein stabilizes the weak *ori*λ–λO–λP complex: size chromatography on Sepharose 4B. ^{14}C-labelled λP protein was preincubated with λdvDNA, λO and DnaJ (open squares); λdvDNA and λO (filled circles); and λdvDNA and DnaJ (open circles) for 10 min at 30°C at the concentration and buffer conditions described by Liberek *et al.* (1988). Subsequently, cross-linking with glutaraldehyde was done as described by Liberek *et al.* (1990) with the exception that the sodium borohydride step was omitted. The 100 μl reaction mixtures were chromatographed through a Sepharose-4B column as described by Liberek *et al.* (1988). The percentage of ^{14}C-labelled λP protein was detected by using a scintillation counter.

in the DnaK requirement of the *in vitro* replication reaction (Zylicz *et al.* 1989; Alfano & McMacken 1989*b*). It has been shown that GrpE protein forms a stable complex with DnaK, and that this complex can be disrupted by the addition of ATP (Zylicz *et al.* 1987). Purified GrpE protein, in the absence of ATP, causes the release of λP protein from DnaK (Osipiuk *et al.* 1993). However, it is also possible to isolate protein complex intermediates by using chemical cross-linking reagents, that contain a monomer of DnaK, a dimer of GrpE, and a dimer or monomer of λP (Osipiuk *et al.* 1993). The ability to isolate such complexes suggests that λP and GrpE interact with DnaK via different domains. It is possible that GrpE induces conformational changes in DnaK which in turn trigger the release of λP from DnaK. Currently, studies of mutual protein–protein interactions between DnaK and λP in the presence or absence of DnaJ, GrpE, or ATP lead us to conclude that GrpE acts as a discrimination factor: DnaK in the absence of GrpE binds equally well to λP or to a λP–DnaJ complex; in the presence of GrpE and DnaJ we observe that seven times more λP is associated with DnaK than in the case where DnaJ was omitted from the reaction (Osipiuk *et al.* 1993). We cannot yet exclude the possibility that the presence of GrpE simply stabilizes the DnaK–λP–DnaJ complex, rather than increasing the affinity of DnaK for the λP–DnaJ complex.

Recently, we were able to show that, in addition to their contribution to the assembly of the appropriate DnaK–λP complex, DnaJ and GrpE also accelerate the ATP-dependent release reaction of DnaK from λP. The intrinsic ATPase activity of DnaK is stimulated up to 50-fold in the joint presence of DnaJ and GrpE; DnaJ specifically accelerates the hydrolysis step ($ATP \rightarrow ADP + P_i$), and GrpE stimulates the release of ADP or ATP from DnaK (Liberek *et al.* 1991*b*). These observations suggest that one of the roles of GrpE and DnaJ is to facilitate the intracellular recycling of DnaK. Whereas the role of DnaK and DnaJ in preprimosomal complex formation has been studied by several laboratories (Dodson *et al.* 1986; Liberek *et al.* 1988; Alfano & McMacken 1989*b*), the effect of GrpE on this process has not been analysed until this paper.

The presence of GrpE allows a reduction in the concentration of DnaK protein required in the λDNA replication reaction, and leads to the formation of a stable intermediate (table 1). Thus one might expect that, in the presence of GrpE, a condition that requires only low DnaK concentrations and increases the specificity of recognition of λP by DnaK, most of the λDNA molecules would begin replicating. To my surprise, however, I found that, despite the fact that the unwinding reaction and λDNA synthesis proceed with high efficiency, most of the λP protein remains associated with λDNA (table 1). Assuming that in both the GrpE-dependent and GrpE-independent systems DnaB helicase needs to be liberated from its association with λP, the results from the GrpE-dependent reaction lead to the interesting possibility that the removal of λP from the primosomal complex is not essential for replication to proceed, rather, it need only be translocated in such a way that it is no longer in direct contact with DnaB. The translocation could be such that λP is retained in the complex with the DNA through its interactions with λO and DnaJ. This complex can only be isolated using cross-linking with glutaraldehyde (figure 2). The direct involvement of such a complex in λDNA replication needs to be determined. However, the existence of the *ori*λ–(λO–λP)–DnaJ complex, after the release of DnaB helicase, could be important for the subsequent reinitiation event taking place during the second round of replication.

3. DISCUSSION

Described above is the mode of action of the DnaK, DnaJ, and GrpE heat shock proteins in λDNA replication. This action is different from the way in which these proteins are understood to work in P1 DNA replication or *oriC*-dependent initiation of *E. coli* DNA replication. The major difference is that, after activation of the initiation factors, RepA in the case of P1 replication (Wickner *et al.* 1991*a*,*b*) and DnaA in the case of *oriC* replication (Hwang *et al.* 1990), the heat shock proteins do not participate directly in subsequent steps of the initiation process. *Ori*P1 replication, like *oriC* replication, does not require heat shock proteins to activate DnaB helicase, probably

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because these replicons use DnaC to transfer DnaB to the origin.

In the case of P1 replication, the RepA protein forms dimers which have a low affinity for binding to *ori*P1 sequences. Activation of RepA occurs through the initial formation of a specific RepA–DnaJ complex. Next, DnaK disassembles RepA dimers into monomers in a reaction that requires ATP hydrolysis. The RepA monomers have a 100-fold higher affinity for *ori*P1 DNA sequences than do the dimers. The action of GrpE, whose involvement *in vivo* and in the crude enzymic fraction *in vitro* assay was recently determined, can be bypassed in the model activation system with purified proteins (Wickner *et al.* 1992). It was also recently shown that the monomeric form of RepA bypasses the requirement of DnaJ, DnaK and GrpE for the *in vitro* replication of *ori*P1 DNA. Therefore the only essential function of these heat shock proteins in P1 replication is in the conversion of RepA from the dimer to the monomer form (Wickner *et al.* 1992).

Another replication system that depends *in vivo* on the presence of DnaK, DnaJ and GrpE is replication of mini-F plasmid DNA (Kawasaki *et al.* 1990). Recently, Kawasaki *et al.* (1992) showed that purified DnaJ protein, but not DnaK (and ATP), dramatically enhances RepE binding to *ori2* DNA sequences. Because *in vivo* replication of mini-F plasmid DNA requires all three heat shock proteins, it is possible that GrpE and DnaK are involved in some later step(s) of mini-F DNA replication.

The involvement of DnaK, DnaJ and GrpE in *oriC* DNA replication remains a mystery. Sakakibara (1988) has shown that the DNA replication defect in bacteria carrying the *dnaK111* mutation is alleviated at the non-permissive temperature by inactivating the *rnh* gene, coding for the RNase H enzyme. This result provides genetic evidence that intact DnaK protein is required at 42°C for the initiation of *E. coli* DNA replication but not for elongation. More recently, it was shown that the *E. coli* DnaA initiation factor, which triggers initiation events by binding to *oriC* sequences, has a high tendency to aggregate (Hwang *et al.* 1990). DnaK protein, in an ATP-dependent reaction, can activate DnaA protein by disassembly of these aggregates (Hwang *et al.* 1990). The roles of DnaJ and GrpE in this reaction are unknown. It is also unclear whether or not the heat shock protein are involved in succeeding steps of *oriC* replication. It has also been shown that DnaA46 mutant protein can be activated in an *oriC*-dependent DNA replication system by pre-incubation with DnaK, ATP and another *E. coli* factor (Hwang & Kaguni 1991), which was recently identified as the *grpE* gene product (J. Kaguni, personal communication). Surprisingly, this reactivation reaction is inhibited in the presence of DnaJ (J. Kaguni, personal communication). It remains unclear what the molecular mechanism of DnaK, GrpE-dependent activation of DnaA46 mutant protein is: is this again a disaggregation reaction? More interestingly, is it possible that the DnaK chaperone could be repairing the tertiary structure of DnaA46 such that this mutant protein is now active in the DNA replication reaction? A similar phenomenon to this latter possibility has been suggested by genetic evidence showing that the overproduction of other chaperones, GroEL and GroES, can overcome the block in the initiation of *E. coli* replication caused by the *dnaA46* mutation (Fayet *et al.* 1986). Although the precise requirement for GroEL and GroES in *oriC*-dependent DNA replication is not known, it has been shown that mutations in *groE* drastically reduce *E. coli* DNA synthesis rates at elevated temperatures (Wada & Itikawa 1984).

It has been suggested that hsp 70-like proteins will bind to hydrophobic domains of other polypeptides (Pelham 1988). Indeed, it is known that one of its members, BiP, binds preferentially to a random mostly hydrophobic stretch of seven amino acids (Flynn *et al.* 1991). There is no particular sequence that is recognized by hsp70, but some experiments support the idea that DnaK binds more efficiently to more unfolded proteins (Langer *et al.* 1992), and that DnaK maintains a bound peptide in its extended conformation (Landry *et al.* 1992). It is still unknown which form of DnaK protein, DnaK, DnaK–ATP, or DnaK–ADP, has a higher affinity for protein substrates.

Summarizing, DnaK alone will bind to a variety of native protein substrates, as well as to unfolded peptides. Recently, it was shown that DnaK binds better to some of its substrates in the presence of DnaJ. This has been demonstrated for RepA protein (Wickner *et al.* 1991*a*,*b*), λP (Osipiuk *et al.* 1993), denatured rhodanese (Langer *et al.* 1992), and the σ^{32} subunit of RNA polymerase (K. Liberek & C. Georgopoulos, unpublished results; Gamer *et al.* 1992). DnaJ could in these cases be a specificity factor that attracts DnaK to the proper protein substrate. It has been shown that the presence of DnaJ stabilizes λP–DnaK, λP–λO, σ^{32}–DnaK, and rhodanese–DnaK complexes even in the presence of ATP (this paper; J. Osipiuk, C. Georgopoulos & M. Zylicz, unpublished results; K. Liberek & C. Georgopoulos, unpublished results; Langer *et al.* 1992). Surprisingly, in the last two examples, ATP even stimulates the binding of DnaK to the DnaJ–substrate complex. This could suggest that, at least for these substrate proteins, DnaK–ATP is the more active form. The role of GrpE is more complex and still not well understood. GrpE could work as a 'discrimination factor' by allowing DnaK to bind with higher affinity to those protein substrates that are already in a complex with DnaJ, as opposed to free protein substrate. This phenomenon has been demonstrated in the case of λDNA replication (Osipiuk *et al.* 1993). Most probably, DnaJ and GrpE perform their functions by modulating the binding of ATP to DnaK, and modulating hydrolysis of ATP catalysed by DnaK protein. Both nucleotide binding (Palleros *et al.* 1992) and ATP hydrolysis (Banecki *et al.* 1992) lead to conformational changes in DnaK. Thus the modulation of ATP binding by GrpE and ATP hydrolysis by DnaJ could be brought about through conformational changes in DnaK. In turn, the different conformations could have different affinities for the various substrates or change affinity for the

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same substrate: one change could stimulate binding, a second change could stimulate release of the substrate from its association with DnaK.

To summarize, in most of the investigated cases, DnaK alone is not able to manifest chaperone activity. Two other auxiliary heat shock proteins, DnaJ and GrpE, are also required. These proteins modulate the binding and release of DnaK from complexes formed with protein substrates. Whether or not both DnaJ and GrpE specificity factors are used in a particular reaction depends on the affinity of DnaK for the particular protein substrate. In one case, the need for DnaJ or GrpE can be overcome by increasing the concentration of DnaK. An example of this is the reactivation of heat-inactivated RNA polymerase (Skowyra *et al.* 1990; D. Skowyra & M. Zylicz, unpublished results). It is still possible that in *E. coli*, in addition to DnaJ and GrpE, there are other specificity factors that help DnaK perform its various functions. The discovery of the DnaJ and GrpE factors was through the selection of *E. coli* mutants which block bacteriophage λDNA replication. Other types of selections may lead to the discovery of different elements that act in concert with DnaK to allow optimal *E. coli* growth.

I record my gratitude to Professor C. Georgopoulos of the University of Geneva for fruitful collaboration over the past 10 years. I thank Dr Jill Zeilstra for her valuable suggestions and corrections of this manuscript. My thanks are also due to Dr S. Wickner, Dr S. J. Kaguni and Dr C. Georgopoulos for unpublished information. I am also grateful to the members of my laboratory: K. Liberek, D. Skowyra, J. Osipiuk, J. Marszalek, Sz. Wojtkowiak, B. Banecki, A. Ziemienowicz, A. Wawrzynow and A. Woloszyk for excellent work and stimulating discussions. This work was supported by grant from State Committee for Scientific Research: KBN (40001/91/01).

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Discussion

R. Jaenicke (*Department of Biophysics and Physical Biochemistry, University of Regensburg, Germany*). With regard to the association of DnaK, can the authors show that there is a dissociation–association equilibrium, and what is the effect on DnaJ on this equilibrium?

M. Zylicz. We have not calculated the equilibrium constant. You have to remember that *in vivo* ATP and DnaJ are present, and the major role of DnaJ is to stabilize the complex and thus overcome the effect of ATP.

R. Jaenicke. Does DnaK on its own show an equilibrium between the monomer and the tetramer?

M. Zylicz. Yes.

M.-J. Gething (*Howard Hughes Medical Institute, University of Texas, Dallas, U.S.A.*). Does DnaJ or GrpE shift the equilibrium between the monomeric and oligomeric forms of DnaK?

M. Zylicz. We were not able to show this, or that ATP affects the ratio of monomers to dimers, unlike the effect reported for the eukaryotic hsp 70 proteins. When DnaJ and GrpE are present the monomeric form of DnaK works well, so that dimers or higher oligomeric forms are not essential.

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R. J. Ellis (*Department of Biological Sciences, University of Warwick, U.K.*). As these *E. coli* proteins are involved in the replication of phage λDNA, is there any evidence that they are also involved in the replication of the *E. coli* DNA?

M. Zylicz. This is not clear. Other authors have shown that the DnaA protein of *E. coli* is an initiation factor in the replication of the bacterial DNA and has a tendency to aggregate; the DnaK protein can disassemble aggregates, so it may be involved. A mutant DnaA protein can be persuaded to work in initiating bacterial DNA replication by the addition of DnaK and GrpE; perhaps this is because of an effect on aggregation, or possibly the DnaK protein changes the conformation of the mutant DnaA protein.

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