Mol Gen Genet (1990) 220:277–282

MGG

Roles of *Escherichia coli* heat shock proteins DnaK, DnaJ and GrpE in mini-F plasmid replication

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Summary. A subset of *Escherichia coli* heat shock proteins, DnaK, DnaJ and GrpE were shown to be required for replication of mini-F plasmid. Strains of *E. coli* K12 carrying a missense mutation or deletion in the *dnaK, dnaJ,* or *grpE* gene were virtually unable to be transformed by mini-F DNA at the temperature (30° C) that permits cell growth. When excess amounts of the replication initiator protein (*repE* gene product) of mini-F were provided by means of a multicopy plasmid carrying *repE*, these mutant bacteria became capable of supporting mini-F replication under the same conditions. However, the copy number of a high copy number mini-F plasmid was reduced in these mutant bacteria as compared with the wild type in the presence of excess RepE protein. Furthermore, mini-F plasmid mutants that produce altered initiator protein and exhibit a very high copy number were able to replicate in strains deficient in any of the above heat shock proteins. These results indicate that the subset of heat shock proteins (DnaK, DnaJ and GrpE) play essential roles that help the functioning of the RepE initiator protein in mini-F DNA replication.

Key words: F factor – Heat shock response – σ^{32} protein – Replication initiator protein – DNA replication

Introduction

Mini-F plasmid is a derivative of the *Escherichia coli* fertility factor (F factor), and exhibits all essential characteristics of the original F replicon including the low copy number (1–2 per host chromosome) (Timmis et al. 1975; Lovett and Helinski 1976). The minimal region required for plasmid replication and its control includes the *repE* gene encoding the initiator protein (RepE protein), an origin of replication (*ori2*), and an incompatibility (*incC*) region (see Kline 1985). The specific interaction between RepE protein and *ori2* is required for initiation of replication (Tokino et al. 1986; Masson and Ray 1986), and the stringent control of plasmid copy number appears to be accomplished at least in part by autogenous and negative regulation of *repE* transcription, and modulation of the RepE protein level by *incC* that also binds the RepE protein (see Kline 1985).

In addition to the plasmid gene products that play highly specific roles, several host factors are probably involved in mini-F replication: DnaE, DNA polymerase; DnaB, DNA helicase; DnaC protein; and DNA gyrase (see Scott 1984). In addition, DnaA protein acts on specific sequences within *ori2* and plays an essential function, presumably in the initiation step (Hansen and Yarmolinsky 1986; Kline et al. 1986; Murakami et al. 1987). The *mafA* gene product appears to modulate mini-F replication through its interaction with *incC* (Wada and Yura 1984).

It has recently been found that transcription of the mini-F *repE* gene is mostly mediated by RNA polymerase containing σ^{32} rather than the usual RNA polymerase containing σ^{70}. Thus, σ^{32} plays a critical role in replication of certain plasmids like F factor in addition to its primary role in the heat shock response (Wada et al. 1986, 1987). As expected from this finding, *ΔrpoH* bacteria lacking σ^{32} cannot support mini-F replication. In contrast, the same σ^{32} deletion strain producing excess RepE protein provided by a multicopy plasmid carrying *repE* permits replication of the mini-F plasmid. These results have been interpreted to mean that the failure of mini-F to replicate in the σ^{32} deletion strain is solely due to the reduced level of RepE protein (see Wada et al. 1987). It has recently been found that one of the major heat shock proteins, DnaK, is essential for mini-F DNA replication (Ezaki et al. 1989). On the other hand, the σ^{32} deletion strain used above is known to produce DnaK and most other heat shock proteins at a very low level (Zhou et al. 1988). In addition, special interest is attached to the role of heat shock proteins in replication of various replicons including plasmids. These considerations prompted us to investigate further the mode of participation of DnaK and other heat shock proteins in mini-F DNA replication.

The DnaK protein is among the most evolutionarily conserved proteins and shows a very high sequence homology (ca. 50%) with eucaryotic HSP70 (Bardwell and Craig 1984). It is required, along with two other heat shock proteins DnaJ and GrpE, for the initiation of replication of bacteriophage λ. Detailed studies both in vivo and in vitro suggest that DnaK and DnaJ play an essential and cooperative role in activating DnaB helicase at the replication origin, and GrpE supports the function of DnaK (for review see Georgopoulos et al. 1990).

All these proteins appear to be essential for bacterial survival and growth at any temperature and play important roles not only in DNA synthesis but in a variety of cellular functions (see Georgopoulos et al. 1990). In the temperature-sensitive *dnaK* mutant examined, the initiation of chro-

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mosomal DNA replication was inhibited at high temperature (Sakakibara 1988). The same set of proteins (DnaK, DnaJ, and GrpE) have recently been implicated in proteolysis (Keller and Simon 1988; Straus et al. 1988) and in the regulation of the heat shock response (D.B. Straus and C.A. Gross, personal communication).

In this paper, it will be shown that not only DnaK but also DnaJ and GrpE proteins are required for mini-F plasmid replication and that these heat shock proteins play essential roles that help functioning of the RepE initiator protein.

Materials and methods

Bacterial strains. Bacterial strains used are all derivatives of *E. coli* K12 and are listed in Table 1. The *dnaK, groEL* and *groES* mutants derived from strain MC4100 were provided by N. Kusukawa. Isogenic pairs of *dnaJ* and *dnaJ*$^+$ strains and of *grpE* and *grpE*$^+$ strains were kindly donated by H. Itikawa and C. Georgopoulos, respectively. KY1603, a temperature-resistant revertant of the *ΔrpoH* mutant lacking σ^{32} but able to grow up to 40° C because of overproduction of GroE protein, has been described (Kusukawa and Yura 1988).

Phage and plasmids. All phage and plasmids used are listed in Table 2. *λdnaK dnaJ* and *λdnaK* phage were gifts of H. Nashimoto. pKV713 was derived from the original mini-F plasmid, pSC138 (Timmis et al. 1975), and served as a standard mini-F. A high copy number (10–15 per chromosome) mini-F plasmid (pKV5110) that essentially consists of *ori2* and *repE*, and its derivatives carrying a *repE* mutation (pKV5111 and pKV5113) will be described elsewhere. pKV718 is a defective mini-F *ori2* plasmid whose replication depends on RepE protein supplied *in trans* by another plasmid carrying *repE*. pKV7090 is a pBR322-based plasmid that produces RepE protein directed by the *trp* promoter (Y. Kawasaki et al. unpublished result). The physical map of mini-F plasmids and mini-F portions of the related plasmids used is shown in Fig. 1.

Table 1. Bacterial strains used

Strain	Genotype	Reference
MC4100	F$^-$ *araD139 Δ(argF-lac)*U169 *rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*	Casadaban (1976)
KY1603	MC4100 *rpoH30*::*kan zhf50*::Tn*10* *suhX401* (λpF13-*PrpoD*hs-*lacZ*)	Kusukawa and Yura (1988)
NRK156	MC4100 *dnaK756 thr-34*::Tn*10*	N. Kusukawa (unpublished)
NRK117	MC4100 *groEL44 zje*::Tn*10*	Kusukawa et al. (1989)
NRK233	MC4100 *groES619 zje*::Tn*10*	Kusukawa et al. (1989)
K7052	*Δ(dnaK dnaJ) thy*	Saito and Uchida (1978)
WJ41	*met*	Itikawa et al. (1986)
WJ45	WJ41 *dnaJ259*	Itikawa et al. (1986)
RB85T	F$^-$ *thr leu thi lacY tonA rpsL supE44 thy*	Tanaka et al. (1985)
MT852	RB85T *thr*$^+$ *dnaJ259 thy*$^+$	M. Wada et al. (1982)
HR1	*lac gal* (λN7N53cItsI-2)	Saito and Uchida (1977)
K2801	HR1 *grpE280*	Ang et al. (1986)
DA15	*thr leu supE pheA*::Tn*10*	Ang et al. (1986)
DA16	DA15 *grpE280*	Ang et al. (1986)

Table 2. Plasmids and phage used

Plasmid or phage	Relevant genotype	Reference or source
pBR322	*bla ori*(pMB9)	Bolivar et al. (1977)
pKV713	*bla ori2 repE incC sopABC*	This work
pKV5110	*bla ori2 repE*	This work
pKV5111	pKV5110 *repE10*	This work
pKV5113	pKV5110 *repE22*	This work
pKV7090	*cat ori*(pMB9) P*trp-repE*	This work
pKV711	pKV7090 *ΔrepE*	This work
pKV718	*bla ori2*	This work
λdnaK	*dnaK*$^+$	Saito and Uchida (1978)
λdnaK dnaJ	*dnaK*$^+$ *dnaJ*$^+$	Saito and Uchida (1978)

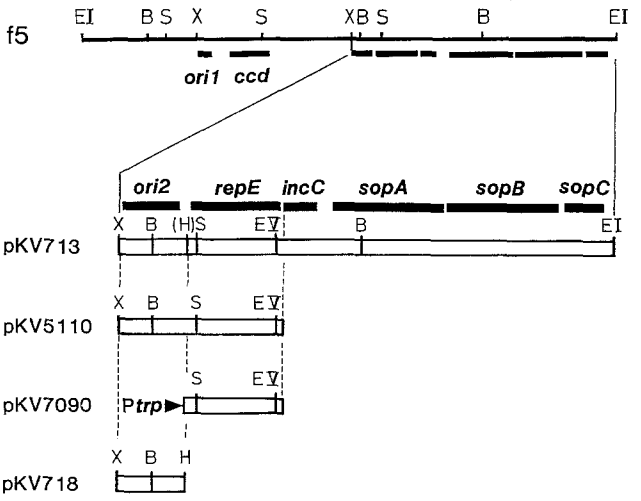

Fig. 1. Physical map of mini-F and mini-F portions of composite plasmids used. The mini-F f5 segment (40.3–49.4 kb of the F coordinate map) is shown at the top. *Filled bars* represent the known genes or regions. In addition to the DNA segments shown here, mini-F plasmids (pKV713, pKV5110 and pKV718) carry an ampicillin resistance gene (*bla*). pKV7090 is a pBR322-based plasmid carrying the mini-F *repE* gene and the chloramphenicol resistance gene (*cat*). EI, *Eco*RI; B, *Bgl*II; S, *Sma*I; X, *Xho*I; EV, *Eco*RV; H, *Hin*fI

Media. L broth contained 10 g Bactotryptone (Difco, Detroit, USA), 5 g yeast extract (Difco) and 5 g NaCl per liter (pH 7.4); 1.2% agar was added for L agar. Thymine (20 μg/ml) was added to L media for growing thymine-requiring strains. Ampicillin and chloramphenicol were used at 20 μg/ml.

Preparation of plasmid DNA. Cells were lysed by lyozyme-EDTA, and plasmid DNAs were prepared essentially as described by Ish-Horowicz and Burke (1981).

Transformation by plasmid DNA. Transformation of bacteria by plasmid was carried out using RbCl-treated cells according to Hanahan (1983). Competent cells and plasmid DNA were mixed, kept on ice for 20 min, treated at 37° C for 3 min, and incubated at 30° C for 90 min before plating the mixture on L agar containing an antibiotic. Plates were incubated at 30° C for 1 to 2 days.

Results

dnaK, dnaJ *and* grpE *mutant strains are defective in mini-F replication*

To examine mini-F plasmid replication in mutants deficient in each of several heat shock proteins, we employed two types of mini-F carrying the ampicillin resistance (*bla*) gene: pKV713, a low copy number plasmid containing a minimum essential region for stable maintenance, and pKV5110, a high copy number plasmid containing a minimum region for replication but deleting *incC*. Several sets of isogenic *E. coli* strains, with a wild-type or mutant allele of each gene, were tested for their ability to be transformed to ampicillin resistance at 30° C, and the results are summarized in Table 3.

In agreement with the results of Ezaki et al. (1989), the temperature-sensitive *dnaK756* mutant deficient in λ DNA replication failed to give any stable transformants when either plasmid was tested. Although the high copy plasmid pKV5110 gave some apparent transformants, they lost the plasmid at high frequencies during subsequent growth in non-selective medium. Strain K7052 with a *dnaK-dnaJ* deletion (Ohki et al. 1989) could not be transformed by mini-F, whereas the same strain lysogenic for *λdnaK-dnaJ* could, as expected. The same strain but lysogenic for *λdnaK* (thus deficient in *dnaJ*) failed to be transformed, suggesting that the *dnaJ* as well as the *dnaK* gene products are required for mini-F transformation. This was further confirmed by testing the temperature-sensitive *dnaJ259* mutants: the mutation in two genetic backgrounds (WJ45 and MT852) clearly affected mini-F replication, while the corresponding isogenic *dnaJ*$^+$ strains (WJ41 and RB85T) were normal (Table 3). Furthermore, the temperature-sensitive *grpE280* mutants (K2801 and DA16) deficient in λ DNA replication were similarly defective in supporting replication of mini-F plasmids. Evidently, all three heat shock proteins, DnaK, DnaJ and GrpE, known to be required for λ DNA replication are also required for mini-F replication.

In contrast, the temperature-sensitive *groEL44 and groES619* mutants deficient in the major heat shock proteins (GroEL or GroES) and in λ phage morphogenesis were shown to be transformed by mini-F at frequencies comparable to those for the wild type at 30° C (Table 3). Mini-F plasmids in the resulting transformants could be stably maintained during cell growth; normal high copy numbers were confirmed with pKV5110 (data not shown). GroE proteins therefore do not seem to be required for mini-F replication.

Excess RepE protein can overcome the defects in mini-F replication

Mini-F plasmid cannot replicate in the *ΔrpoH* strain (KY1603) lacking σ^{32} required for the expression and regulation of the heat shock proteins (Table 3). However, the same strain carrying a multicopy plasmid pKV7090 that allows transcription of *repE* from the *trp* promoter permits mini-F replication because excess RepE protein is supplied *in trans*. It has been shown that these strains produce very little DnaK protein (less than 1% of wild type) (Zhou et al. 1988). In an attempt to resolve the apparent contradiction between these results and those of Ezaki et al. (1989) dealing with the DnaK requirement in mini-F replication, we thought of the possibility that DnaK might be dispensable for mini-F replication in the presence of excess RepE protein. Thus the effect of the *repE*-expressing plasmid (pKV7090) on mini-F replication was examined in the *dnaK, dnaJ* and *grpE* mutants.

As expected, the low copy mini-F plasmid (pKV713) could replicate in any of the *dnaK, dnaJ* and *grpE* mutants tested, provided that they carried pKV7090 (Table 4). The

Table 3. Transformation efficiencies of plasmids

Strain	Defect in heat shock gene	Plasmid			
		pKV713	pKV5110	pKV5111	pKV5113
MC4100	–	0.94	0.78	(1.0)	3.9
NRK156	*dnaK756*	<0.0008	(0.0084)	(0.22)	3.2
NRK117	*groEL44*	0.12	0.17	n.t.	n.t.
NRK233	*groES619*	1.8	4.7	n.t.	n.t.
K7052	*ΔdnaK-dnaJ*	<0.01	<0.002	(0.14)	0.82
K7052 (*λdnaK*)	*ΔdnaJ*	<0.04	<0.009	(0.087)	2.7
K7052 (*λdnaK dnaJ*)	–	0.33	0.12	(0.29)	0.36
WJ41	–	0.26	0.10	(0.15)	0.12
WJ45	*dnaJ259*	<0.0004	<0.0006	(0.22)	0.081
RB85T	–	0.41	0.94	(0.89)	0.12
MT852	*dnaJ259*	<0.002	<0.003	(0.13)	0.24
HR1	–	0.12	0.42	(0.032)	0.12
K2801	*grpE280*	<0.001	<0.0004	(0.019)	0.074
DA15	–	0.28	0.92	(0.03)	1.0
DA16	*grpE280*	<0.0001	<0.00005	(0.049)	0.12
KY1603	*ΔrpoH*	<0.001	<0.0003	0.15	0.25

Transformation efficiencies at 30° C relative to those of pBR322 are presented. n.t., not tested. Values in parentheses refer to small or flat colonies that could not stably maintain the mini-F plasmid by subsequent tests

Table 4. Transformation of strains producing excess RepE protein

Strain (carrying pKV7090)	Defect in heat shock gene	Plasmid	
		pKV713	pKV718
MC4100	–	n.t.	3.2×10^4
NRK156	*dnaK756*	4.3×10^4	1.6×10^3
K7052	*ΔdnaK-dnaJ*	3.8×10^3	1.8×10^4
K7052(*λdnaK*)	*ΔdnaJ*	6.4×10^3	3.4×10^3
K7052(*λdnaK dnaJ*)	–	n.t.	1.3×10^4
WJ41	–	n.t.	4.4×10^4
WJ45	*dnaJ259*	6.2×10^3	6.5×10^4
RB85T	–	n.t.	5.6×10^4
MT852	*dnaJ259*	1.5×10^4	3.9×10^4
HR1	–	n.t.	1.1×10^3
K2801	*grpE280*	1.7×10^4	1.2×10^3
DA15	–	n.t.	1.5×10^4
DA16	*grpE280*	2.2×10^3	3.4×10^4
KY1603	*ΔrpoH*	1.1×10^4	9.9×10^3

Each strain carrying pKV7090 that expresses *repE* from the *trp* promoter was transformed, by the plasmid indicated, to ampicillin resistance at 30° C. Frequencies of transformants obtained per microgram DNA are presented. n.t., not tested

apparent increase in transformation frequency brought about by pKV7090 was at least 1000-fold. The same result was obtained when an "*ori2* plasmid" (pKV718) containing *ori2* but not *repE* was introduced into strains harboring pKV7090 (Table 4; see Fig. 2A); strains harboring pKV711 (same as pKV7090 but deleted for *repE*) gave no transformants (data not shown). Thus the requirement for DnaK, DnaJ, and GrpE proteins in mini-F replication is not absolute and can be removed by providing excess RepE protein. The results suggest that these heat shock proteins exert their function by promoting the function (not the synthesis; see below) of RepE protein by some unknown mechanism.

DnaK, DnaJ and GrpE proteins enhance the level of plasmid replication

To examine whether the *dnaK, dnaJ,* or *grpE* mutation affects the level of mini-F replication in the presence of excess RepE protein, we determined the copy number of the *ori2* plasmid pKV718 in the various strains obtained above, pKV7090 serving as an internal reference; replication of the latter plasmid was not affected by any of the heat shock protein mutants employed (Fig. 2B). The density of the pKV718 band relative to that of pKV7090 for each mutant was normalized to the wild-type value (Fig. 2C). We have confirmed that the amounts of RepE protein produced in these mutants as determined by immunoblotting were no less than that in the wild type (data not shown). The copy number of pKV718 thus obtained was found to be low (less than 0.3) in most of the mutants tested including strains with the genotype *ΔdnaK-dnaJ, ΔdnaJ, dnaJ259,* or *grpE280*. The *dnaK756* mutant showed a somewhat higher value (0.44) which is consistent with the apparent partial capacity of this strain to support replication of the high copy mini-F plasmid (pKV5110) (Table 3). It should be noted that the *ΔdnaK-dnaJ* mutant deficient in both DnaK and DnaJ proteins supported *ori2* plasmid replication to the same extent as did the *dnaK* or *dnaJ* mutant (Fig. 2C). These results clearly indicate that a deficiency in any of the set of proteins, DnaK, DnaJ and GrpE, reduces the

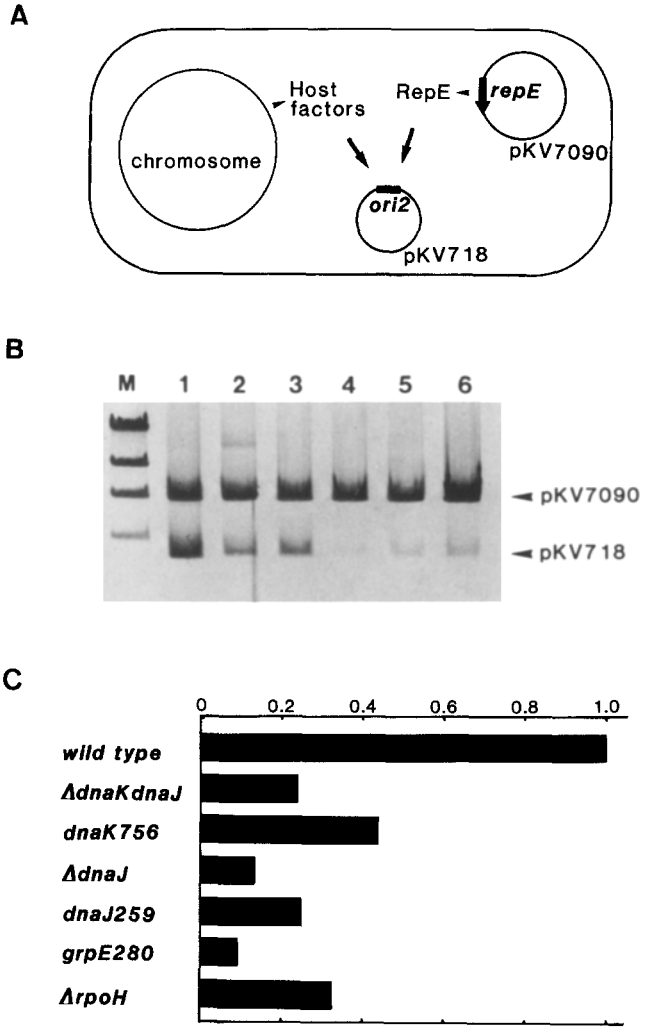

Fig. 2A–C. Replication of *ori2* plasmid (pKV718) in the presence of excess RepE protein. Strains harboring pKV7090 that expresses *repE* were transformed by pKV718, selecting for ampicillin resistance at 30° C. pKV7090 provides excess RepE proteins to be utilized for replication of pKV718, as illustrated in **A**. The resulting transformants were grown in the presence of both chloramphenicol and ampicillin at 30° C. Plasmid DNAs were extracted from early stationary phase cells, digested with *Eco*RI, and analyzed by agarose (1%) gel electrophoresis **B**. M, λ DNA digested with *Hin*dIII; lane 1, MC4100 (wild type); lane 2, K7052 (*ΔdnaKdnaJ*); lane 3, NRK156 (*dnaK756*); lane 4, K7052 (*λdnaK*); lane 5, MT852 (*dnaJ259*); lane 6, DA16 (*grpE280*). DNA bands obtained in **B** were scanned with a densitometer (Biomed Instruments, Calif, USA), and the ratio of pKV718 DNA to pKV7090 DNA was normalized to the wild-type value. Averages of at least five samples are presented **C**

cellular capacity for mini-F replication (and thus reduces copy number) in the presence of excess RepE protein. They further suggest that all these heat shock proteins play specific and cooperative roles in enhancing the level of mini-F replication.

Mini-F mutants that can replicate in dnaK, dnaJ, *and* grpE *mutant bacteria*

We have recently isolated and characterized mutant mini-F plasmids (pKV5111 and pKV5113) that can replicate in the absence of σ^{32}; they can transform the *ΔrpoH* strain

(KY1603) at high frequencies (Y. Kawasaki et al. unpublished results; see Table 3, bottom line). These mutant plasmids carry a mutation in the coding region of *repE* and produce altered RepE protein in large amounts, resulting in a very high copy number. One of them (pKV5111) cannot form stable transformants with the *rpoH*$^+$ strains presumably because of the extremely high copy number.

These mutant mini-F plasmids were tested to see if they can transform the *dnaK, dnaJ,* and *grpE* mutant hosts to ampicillin resistance at 30° C (Table 3). Indeed, transformant colonies appeared at frequencies much higher than those for the wild-type plasmid (pKV5110), though in the case of pKV5111, they were unstable and lost the plasmid at high frequencies during further growth. In contrast, pKV5113 gave stable transformants with all the strains tested at high but somewhat variable frequencies (Table 3). Thus the requirement for DnaK, DnaJ and GrpE proteins in mini-F replication could be bypassed at least partially by these mini-F *repE* mutations. These results are consistent with those presented above on the effects of excess wild-type RepE protein on mini-F replication in the heat shock protein mutants, and support the view that DnaK, DnaJ, and GrpE proteins play essential roles in mini-F replication by assisting the function of RepE protein in the initiation step.

Discussion

The roles of the *E. coli* heat shock proteins DnaK, DnaJ and GrpE in DNA replication and other cellular processes have been elucidated by several recent lines of work. In λ phage replication, sequential protein assembly and disassembly has been shown to take place at the origin (*ori*λ) prior to the initiation of replication (see Georgopoulos et al. 1990). DnaJ and DnaK proteins specifically bind to the *ori*λ-λO-λP-DnaB complex to help the disassembly of λP from the complex, resulting in the activation of DnaB helicase. GrpE potentiates the action of DnaK in this process in vivo, but is dispensable in the in vitro reaction when the concentration of DnaK is sufficiently high. Thus DnaK, DnaJ and GrpE work cooperatively and permit DnaB helicase to initiate local unwinding of the *ori*λ DNA. In *E. coli* chromosomal replication, genetic evidence implicates DnaK protein as involved in the inititation step, but its mode of action remains unknown (Sakakibara 1988).

We have shown that not only DnaK, as reported previously (Ezaki et al. 1989), but also DnaJ and GrpE take part in mini-F DNA replication. On the other hand, these heat shock proteins were found to be dispensable when excess RepE protein is present in the cell, although they do enhance the replication efficiency significantly under these conditions. In this situation, the three heat shock proteins appear to play specific but cooperative roles in increasing the copy number. Moreover, certain *repE* mutations that enhance the replication capacity can also suppress the requirement for the same set of proteins. In addition to the roles in DNA replication, DnaK, DnaJ and GrpE proteins have recently been implicated in modulation of energy-dependent proteolysis (Straus et al. 1988; Keller and Simon 1988) and the synthesis and degradation of the heat shock sigma factor, σ^{32} (D.B. Straus and C.A. Gross, personal communication). These results lead us to propose that the set of heat shock proteins somehow assist the functioning of RepE protein in the initiation of mini-F DNA replication.

Although the mechanisms underlying the present observations remain obscure, it may be worth considering two distinct possibilities, though they are by no means mutually exclusive. First, the set of heat shock proteins may be involved in activation or stabilization of RepE protein before its binding to *ori2* DNA. It has been proposed that RepE protein undergoes structural modification or processing to gain its initiator activity (Trawick and Kline 1985; Womble and Rownd 1987). The set of heat shock proteins might be involved in such structural changes, for example, dimerization, phosphorylation, or proteolytic cleavage. The known activities and properties of DnaK, DnaJ and GrpE proteins would be consistent with their involvement in any of these processes. Second, they might help assembly of RepE protein into the preinitiation complex (or disassembly of some replication protein away from the complex) at *ori2* DNA in a manner similar to that for λ DNA replication, even though the actual mechanism may be quite different.

With respect to other plasmids, we have found that the copy number of mini-Rts1 (derived from Rts1) is reduced in the *dnaK, dnaJ* and *grpE* mutants. Also, mini-P1 (derived from phage P1) cannot be transformed efficiently into *dnaK, dnaJ* or *grpE* mutants (K. Tilly, personal communication; Y. Kawasaki, unpublished results). On the other hand, multicopy plasmids such as pBR322 or pACYC184, and pSC101, can replicate in the *dnaK* deletion strain (Ezaki et al. 1989). pBR322 and pSC101 can transform the *dnaJ* and *grpE* mutants at normal frequencies. Mini-R6K carrying a minimum essential region of R6K also replicates apparently normally in these mutant bacteria (Y. Kawasaki, unpublished results). It thus appears that the requirement for DnaK, DnaJ and GrpE proteins in DNA initiation is limited to F and some closely related plasmids.

Wada et al. (1986, 1987) have found that mini-F cannot replicate normally in *rpoH* mutants deficient in σ^{32} and that *repE* is mainly transcribed by RNA polymerase containing σ^{32}. Taking these results together with the present results on the heat shock proteins, we conclude that σ^{32} has dual functions in the replication of the F and mini-F plasmids. On the one hand, σ^{32} controls *repE* transcription and thus controls synthesis of RepE protein. On the other hand, it controls transcription of *dnaK, dnaJ* and *grpE* and therefore production of the corresponding heat shock proteins required for proper functioning of RepE protein. Further studies on the mechanisms of involvement of these proteins and their interaction with other host factors and the RepE initiator protein should provide basic information on mini-F replication and its regulation.

Acknowledgements. We are grateful to C. Georgopoulos, H. Itikawa, N. Kusukawa, and H. Nashimoto for kind gifts of bacterial strains and phage, and to Y. Yoshioka, M. Mihara and K. Tanaka for technical assistance. We thank S. Hiraga for communicating results prior to publication. This work was supported in part by grants from the Ministry of Education, Science and Culture, Japan.

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Communicated by M. Sekiguchi

Received August 20, 1989