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SHORT COMMUNICATION

Disassembly of the Coliphage λ Replication Complex Due to Heat Shock Induction of the *groE* Operon

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We have found previously that, in contrast to the free O initiator protein of λ phage or plasmid rapidly degraded by the *Escherichia coli* ClpP/ClpX protease, the λO present in the replication complex (RC) is protected from proteolysis. In amino acid-starved *E. coli relA* cells, a temperature shift from 30 to 43° did not affect RC integrity, as judged from the unchanged level of stable λO observed; however, the same temperature shift in a complete medium resulted in the decay of this λO fraction, which suggested disassembly of the RC. Examination of this phenomenon revealed that for λ RC disassembly, heat shock induction of the *groE* operon, coding for molecular chaperones of the Hsp60 class, is indispensable. Heat shock induction of the *groE* operon present on a multicopy plasmid inhibited the growth of infecting phage.

Replication of phage λ DNA in *Escherichia coli* occurs according to the θ (circle-to-circle) mode at early times after infection and is switched later to the σ (rolling-circle) mode. The plasmids derived from phage λ replicate exclusively according to the θ mode, which seems to be identical with the phage λ DNA early replication mode (for recent review see Ref. *1*). The λ phage or plasmid codes for two replication proteins, O and P. λO binds to the *origin* of replication, oriλ, situated in the middle of the *O* gene, and λP, complexed with bacterial DnaB helicase, binds to λO (*2*). The stable fraction of λO initiator protein remaining after degradation of its excess by ClpP/ClpX protease stands for the λO present in the replication complex (RC) (*3, 4*). In the pathway of RC assembly the λO stabilization occurs at the step of formation of the λO–λP–DnaB preprimosome (*5*), before the DnaA-regulated transcriptional activation of the origin of replication, oriλ (*6*). Since λP does not seem to leave the preprimosome due to the action of a complete set (DnaK, DnaJ, and GrpE) of Hsp70 chaperone proteins *in vitro* (*7*) (a situation more closely resembling *in vivo* conditions), we describe this process as rearrangement of preprimosomal constituents. Other authors usually describe it as disassembly, because it was previously demonstrated that in incomplete systems (lacking GrpE) λP leaves the complex after the action of DnaK and DnaJ proteins (*8, 9*). Preprimosomal rearrangement results in the release of DnaB helicase from λP inhibition. It has been also suggested that the rearrangement is coupled with the DnaA-regulated transcription required for activation of oriλ (*10*). The preprimosome would be inserted between the λ DNA complementary strands, transiently separated by transcription, by the action of Hsp70 chaperones, recently called force-generating proteins (*11*). Such insertion of the DnaB helicase-containing structure is a prerequisite for the formation of replication forks. The once-assembled RC is a stable, λO-containing structure inherited at each round of replication by one of two daughter λ plasmid copies (*12, 13*). For the initiation of a next round of replication by the old RC the functions of RNA polymerase, DnaA, and chaperone proteins of the Hsp70 class are required in amino acid-starved *E. coli relA* (relaxed) cells (*13, 14*), as well as in wild-type cells in a complete medium (A. Węgrzyn, G. Węgrzyn, A. Herman, K. Taylor, submitted). This suggests that the DnaA-regulated transcriptional activation of oriλ and chaperone-mediated insertion between the λ DNA strands take place also in the case of initiation performed by the old RC. However, in contrast to the initiation of replication starting from the naked oriλ, the λP function required for RC assembly and inactivated at 43° by the ts1 mutation is dispensable in the replication initiated by the old RC (*14*).

In amino acid-starved *E. coli relA* cells the λ plasmid DNA replication occurs only due to the activity of RC assembled before the onset of amino acid starvation. The assembly of a new RC cannot occur because the λO initiator is not synthesized (*3*) and the excess of previously synthesized λO has been degraded by ClpP/ClpX

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TABLE 1

Escherichia coli K-12 Strains

Strain	Genotype	Reference
MG1655	Wild type	*26*
SC122	*lac trp mal str supC*(ts)	*27*
K165	As SC122 but *rpoH*(am)	*27*
C600	*supE*44 *hsdR thi*-1 *thr*-1 *leuB6 lacY*1 *tonA*21	*28*
BM237	As C600 but *grpE*280 *zfh::Tn10*	*10*
BM238	As C600 but *dnaK*756 *zaa::Tn10*	*10*
BM239	As C600 but *dnaJ*259 *thr::Tn10*	*10*
BM270	*groEL*44	*29*
BM271	*groES*619	*29*
SG22097	*clpP::cm clpX::kan*	*16*

protease (*4, 12, 15*), the enzyme specifically acting on λO (*16, 17*). In these conditions the temperature shift from 30 to 43° did not affect RC integrity, as judged from the unchanged level of stable λO observed (*14*). However, in the case of wild-type bacteria (*E. coli* strains are listed in Table 1) harboring wild-type λ plasmid (plasmids are described in Table 2), the same temperature shift in a complete medium resulted in the decay of this λO fraction, which suggested disassembly of RC (Fig. 1A). This process did not concern simply the growth at 43°, since the stable λO appeared in the same bacteria grown overnight at this temperature (Fig. 1B). We concluded, therefore, that RC disassembly may result from induction of heat shock protein synthesis which should be possible only in cells growing in a complete medium. Indeed, the temperature shift did not cause any decay of the originally stable λO in the *rpoH* (*htpR*) mutant (Fig. 1C); the *rpoH* gene codes for the σ^{32} subunit of RNA polymerase specific for the heat shock regulon. A distinct decay of this λO fraction occurred in the otherwise isogenic *rpoH*$^+$ cells (Fig. 1D).

The λ plasmid replication seems to occur due to RCs assembled with the help of constitutively synthesized Hsp70 (DnaK) chaperone proteins, because there is no reason for induction of their synthesis in λ plasmid-harboring cells. Therefore, it was possible that, when present at high levels after a temperature shift, the Hsp70

TABLE 2

Plasmids

Plasmid	Description	Reference
pKB2	Wild type λ plasmid bearing a kanamycin-resistance gene	*30*
pAS1	Wild type λ plasmid bearing a tetracycline-resistance gene	*4*
pCB104	Wild type λ plasmid bearing a chloramphenicol-resistance gene	*31*
pAW6	As pCB104 but bearing the mutation πA66 which allows the plasmid to replicate in *dnaK*756, *dnaJ*259, and *grpE*280 strains	*14*
pOF39	The pBR325-derivative plasmid bearing the wild-type *groE* operon	*32*
pSK20	The pBR322-derived replicon bearing the *clpP–clpX* operon under the control of the λp_L promoter	*16*

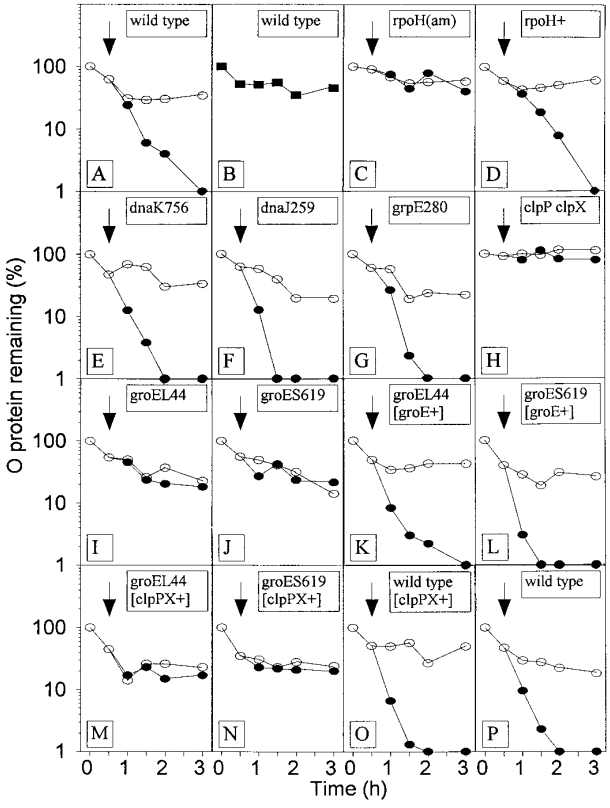

FIG. 1. λO protein decay in *E. coli* strains harboring λ plasmids at 30° (open circles), after temperature shift from 30 to 43° at the time indicated by the arrow (filled circles), and at 43° (filled squares on B; bacteria were grown overnight at 43° and the whole experiment from labeling to samples' withdrawal was performed at this temperature). (A) wild type (MG1655)/pKB2, (B) wild type (MG1655)/pKB2, (C) *rpoH*$^-$ (K165)/pKB2, (D) *rpoH*$^+$ (SC122)/pKB2, (E) *dnaK*756 (BM238)/pAW6, (F) *dnaJ*259 (BM239)/pAW6, (G) *grpE*280 (BM237)/pAW6, (H) *clpP*::*cm clpX*::*kan* (SG22097)/pAS1, (I) *groEL*44 (BM270)/pCB104, (J) *groES*619 (BM271)/pCB104, (K) *groEL*44 (BM270)/pOF39/pCB104, (L) *groES*619 (BM271)/pOF39/pCB104, (M) *groEL*44 (BM270)/pSK20/pCB104, (N) *groES*619 (BM271)/pSK20/pCB104, (O) wild type (MG1655)/pSK20/pCB104, and (P) wild type (MG1655)/pCB104. The experiments were performed as described previously (*3, 5, 14*). Briefly, *E. coli* strains harboring a λ plasmid were grown at 30° (in all experiments but that presented on B) in minimal medium 2 (*33*) to A_{575} = 0.2 and then labeled with Tran[^{35}S]-label (ICN Radiochemicals) for 20 min. The label was chased by addition of excess of unlabeled L-methionine and L-cysteine (Time 0). After 30 min, half of each culture was transferred to 43° and the second half remained at 30° (it concerned all experiments but that presented on B). The samples were withdrawn at indicated times and precipitated with ice-cold acetone, and after centrifugation the pellets were frozen at −20°. After thawing, immunoprecipitation with anti-λO serum (*5*), SDS–polyacrylamide gel electrophoresis, and autoradiography were performed as described previously (*3*). ^{35}S-labeled λO protein visualized by autoradiography was quantitated by densitometry using UVP EASY Enhanced Analysis System (Cambridge, England).

system was engaged in RC disassembly. However, the experiments performed with *dnaK*756 (Fig. 1E), *dnaJ*259 (Fig. 1F), and *grpE*280 (Fig. 1G) mutants clearly demonstrated that this was not the case.

The other *E. coli* system of chaperone proteins is Hsp60, composed of two proteins, GroEL and GroES. They are engaged in the λ phage head morphogenesis (*18*); no other involvement in the λ phage intracellular development or λ plasmid replication is known. In contrast to the preceding experiments, in both mutants, *groEL* (Fig. 1I) and *groES* (Fig. 1J), the level of the stable λO fraction remained unchanged after the temperature shift. The decay of this λO fraction was observed again, however, when a plasmid carrying the *groE* operon has been introduced into the *groEL* (Fig. 1K) or *groES* (Fig. 1L) mutant. This set of experiments demonstrated that the Hsp60 system is involved (most probably by disassembling RC) in a process leading to the decay of the stable λO fraction which occurs after the temperature shift up.

In a separate experiment we found that the ClpX/ClpP protease is responsible for the decay of λO protein previously protected from this enzyme by other constituents of RC. If any other protease were engaged in the decay of this particular λO fraction, the λO proteolysis should be observed after heat shock of the ClpX/ClpP protease-devoid mutant. That is not the case, as shown in Fig. 1H. The λO decay after the start of isotope chasing (Time 0), representing ClpX/ClpP-mediated proteolysis of an excess of synthesized free λO, occurred in the *groE* mutants (compare Figs. 1I and 1J with 1H) as usual, indicating that they are not defective concerning this enzyme, thus ruling out a possibility that the functional GroE system is required for the ClpX/ClpP activity.

Since the *clpP–clpX* operon belongs to the heat shock regulon, there was a possibility that increased level of the ClpX/ClpP protease is primarily responsible for the decay of stable λO fraction in *groE*$^+$ cells after a temperature shift up, and this process does not work in *groE* mutants for some unknown reason. However, introduction of the *clpP–clpX* operon-containing multicopy plasmid (pSK20) to the λ plasmid-harboring *groE* mutants (resulting in a substantial increase of ClpP and ClpX proteins, as revealed on our Coomassie-stained SDS–polyacrylamide electrophoregrams; results not shown) did not affect substantially the proteolysis-resistant λO fraction which was observed both at 30° and after raising temperature to 43° (Figs. 1M and 1N). This supported our conclusion that the stability of λO fraction in the *groE* mutants is not due to an abnormally low level of ClpX/ClpP protease. The presence of the stable λO fraction in ClpX/ClpP-overproducing cells at 30° (Fig. 1O) at the level revealed in control experiments (Figs. 1A and 1P), taken together with an uninhibited λ plasmid or phage replication in the presence of an excess of this enzyme (*4*), is again compatible with the idea of identity of this fraction with λO protected from proteolysis by other constituents of RC.

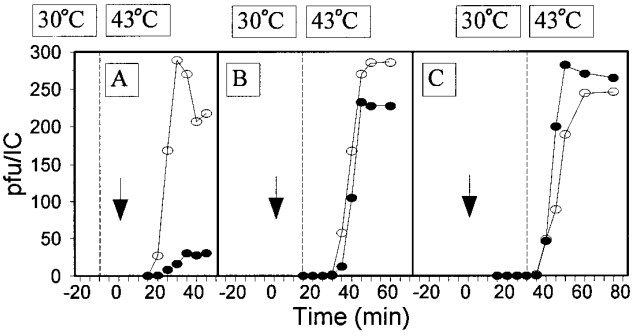

FIG. 2. Effect of GroEL and GroES overproduction on the growth of phage λ*clb2.* One-step growth experiments in *E. coli* MG1655 (empty circles) and MG1655/pOF39 (filled circles) cells. The temperature shift from 30 to 43° (indicated by dashed vertical line) was performed (A) 10 min before, (B) 15 min after, or (C) 30 min after phage addition. The arrows indicate the time of infection. The results are presented as plaque forming units (pfu) per infective center (IC). The experiments were performed as described previously (*4, 10*) but NaN_3 was avoided and the phage adsorption, shortened to 2.5 min, was followed directly by a 2.5-min incubation with anti-λO serum and 1000-fold dilution in LB medium (Time 0).

The results presented strongly support the idea that in the observed phenomenon the ClpX/ClpP protease acts on the λO, which has been released from RC (stable λO fraction) by the action of the GroE system induced by the temperature shift up.

Heat shock does not affect much the replication of λ plasmid occurring in wild-type cells (results not shown). Probably the disassembled RCs are replaced by the RCs assembled *de novo.* After a transient wave of higher concentration/activity of GroE chaperone proteins the new RCs may function unhampered at a steady state specific for 43°, as may be deduced from Fig. 1B. It seems that the same concerns the replication of the phage λ; the yield of phage progeny obtained after thermoinduction of a *c*Its prophage is comparable to that obtained without heat shock, after the action of UV or mitomycin C on λ$^+$ lysogens. This is the reason why we decided to study phage λ development in bacteria harboring a multicopy plasmid containing the *groE* operon.

The presence of the *groE* operon-containing multicopy plasmid (pOF39) did not affect the growth of host bacteria before or after the temperature shift from 30 to 43° (results not shown). The one-step growth experiments revealed that, when the phage λ infection was preceded by the heat shock-provoked induction of pOF39 plasmid-harboring cells, the phage growth was considerably inhibited (Fig. 2A). Since the peak of chaperone proteins usually appears 10–15 min after heat shock, in this particular experiment it coincides with the time of RC assembly at *ori* of the infecting λ DNA molecule. No inhibition of phage growth was observed in control experiments with the vector plasmid (pBR325)-harboring bacteria con-

taining only the chromosomal *groE* operon (results not shown). When the temperature shift was performed 15 or 30 min after phage addition, the presence of pOF39 plasmid had little effect on the kinetics of phage growth (Figs. 2B and 2C), showing that the overproduction of GroEL and GroES proteins does not practically interfere with late stages of λ phage development.

The GroEL and GroES proteins can jointly deaggregate and reactivate the heat-treated RNA polymerase *in vitro* (*19*). Also the heat shock-provoked protein aggregates in *E. coli* cells (*20*) disappear largely due to their action, as suggested by the observation of *groE* mutants (A. Taylor, personal communication). Here we present for the first time the evidence that the Hsp60 (GroEL) system is engaged in an *in vivo* disassembly of a highly organized protein structure, the λ replication complex, under stress caused by temperature shift. This structure is dispensable for cell survival and it may be that its components are reutilized for the assembly of the host replication complex. It is possible that the observed phenomenon represents a general strategy of a cell endangered by unfavorable environmental conditions. If this phenomenon is general, the results obtained may provide a molecular basis for a new approach to the treatment of viral infections.

Current models by which molecular chaperones facilitate protein folding/assembly point to the sequence of action which starts with the Hsp70 (DnaK) class and is followed by the Hsp60 (GroEL) class (*18, 21*). In some systems even a "handoff" between Hsp70 (DnaK) and Hsp60 (GroEL) was suggested (*22, 23*). It is intriguing that in our case the host chaperone classes are utilized in the same sequence, at first for λ RC assembly, then for disassembly of this structure under stress conditions. Two members of the Hsp100 class, ClpA and ClpX, provide substrate specificity to the ClpP protease (*24*). The ClpX chaperone protein alone may cause deaggregation of the λO aggregates *in vitro* (*25*), but the complex ClpP/ClpX causes specific proteolysis of λO. The possible handoff between GroEL and the Clp chaperone/protease system in the disassembly of RC awaits further studies.

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