Induction of the Heat Shock Regulon of *Escherichia coli* Markedly Increases Production of Bacterial Viruses at High Temperatures

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Production of bacteriophages T2, T4, and T6 at 42.8 to 44°C was increased from 8- to 260-fold by adapting the *Escherichia coli* host (grown at 30°C) to growth at the high temperature for 8 min before infection; this increase was abolished if the host *htpR* (*rpoH*) gene was inactive. Others have shown that the *htpR* protein increases or activates the synthesis of at least 17 *E. coli* heat shock proteins upon raising the growth temperature above a certain level. At 43.8 to 44°C in T4-infected, unadapted cells, the rates of RNA, DNA, and protein synthesis were about 100, 70, and 70%, respectively, of those in T4-infected, adapted cells. Production of the major processed capsid protein, gp23*, was reduced significantly more than that of most other T4 proteins in unadapted cells relative to adapted cells. Only 4.6% of the T4 DNA made in unadapted cells was resistant to micrococcal nuclease, versus 50% in adapted cells. Thus, defective maturation of T4 heads appears to explain the failure of phage production in unadapted cells. Overproduction of the heat shock protein GroEL from plasmids restored T4 production in unadapted cells to about 50% of that seen in adapted cells. T4-infected, adapted *E. coli* B at around 44°C exhibited a partial tryptophan deficiency; this correlated with reduced uptake of uracil that is probably caused by partial induction of stringency. Production of bacteriophage T7 at 44°C was increased two- to fourfold by adapting the host to 44°C before infection; evidence against involvement of the *htpR* (*rpoH*) gene is presented. This work and recent work with bacteriophage λ (C. Waghorne and C. R. Fuerst, *Virology* 141:51-64, 1985) appear to represent the first demonstrations for any virus that expression of the heat shock regulon of a host is necessary for virus production at high temperature.

Exposure of living organisms to a sudden increase in temperature triggers a heat shock response whereby the expression of a specific set of genes encoding the heat shock proteins is markedly stimulated, thus providing a homeostatic adaptation to this stress; the effect has been demonstrated in all organisms examined (2).

Workers in this laboratory (74; M. F. Mowrey, Ph.D. thesis, University of Rochester, 1977) confirmed an unpublished observation by S. E. Luria (personal communication) that adaptation of bacteria to growth at high temperature before infection greatly increased the production of T-even bacteriophage (bacterial viruses) at that temperature. With the more recent developments in understanding heat shock, specifically the availability of *Escherichia coli* mutants (*htpR* = *hin* = *rpoH*) defective in regulation of expression of the heat shock genes (27, 45, 46, 70, 78), it became possible to test whether the adaptation effect on phage production is due to expression of the heat shock regulon of *E. coli*. We present evidence here that expression of the *E. coli htpR* gene is responsible for the adaptation effect with phage T2, T4, and T6 but not with the unrelated phage T7, whose production is increased two- to fourfold by adaptation. We show that the heat shock protein GroEL is responsible for about half of the adaptation effect with T4. The possible roles of other heat shock proteins in the adaptation effect are discussed. (See Table 2, footnotes *e* and *g*, for definitions of unadapted and adapted cells.)

Recently, Waghorne and Fuerst (73) showed that both lytic and lysogenic production of bacteriophage lambda is

greatly increased at high temperature by expression of the *E. coli htpR* gene.

MATERIALS AND METHODS

Phage and bacteria. The wild-type phage used were T2H, T4D, T6, and T7. T4D *amH11* (gene 23) and *amE303* (gene 24) were from Bill Wood. T4D *amHA9* (gene IPIII) was from Lindsay Black. The double mutant *amH11 amE303* was constructed in this laboratory. Since T7 tends to suffer spontaneous deletions (66), we tested the integrity of our stock (obtained from Studier in 1978) by restriction enzyme analysis of the DNA. The *HpaI* cleavage pattern (data not shown) matched that of T7 Studier (66) and of a sample of T7 DNA obtained from David Hinkle. Thus our T7 appears to be the authentic wild type. The bacteria and plasmids used are shown in Table 1; wild-type *E. coli* B (our stock no. JW42) was obtained from R. Epstein in 1962. It routinely produces a few percent long filamentous forms.

Strains SC122 and K165 are supposedly isogenic except in the *htpR* gene; however, in our hands SC122 differs in two respects from K165 and K166(pFN97). (i) SC122 is far more motile than the other two when viewed in the microscope; F. C. Neidhardt has observed this independently (personal communication). (ii) SC122 adsorbs T2, T4, and T6 poorly compared with the other two strains, especially at 42.8°C (see Table 3). The adsorption of T2 to SC122 was enough better than that of T4 and T6 so that T2 gave the most reliable infections. Since all three cultures were grown up to the same optical absorbance before starting the experiment in Table 3, they all presumably contained the same cell-mass concentration. We believe cell-mass concentration represents a more reliable basis for comparison of phage production among the different strains than does concentration of colony formers, where the average cell size may differ from strain to strain. The concentrations of colony formers per ml

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TABLE 1. *E. coli* strains and plasmids

Strain or plasmid	Relevant genotype or phenotype	Source or reference
<i>E. coli</i>		
B	Wild-type <i>E. coli</i> B	This laboratory
SC122	<i>htpR</i> ⁺ <i>supC(ts)</i> <i>trp(am)</i>	F. Neidhardt (12)
K165	<i>htpR(am)</i> derivative of SC122	F. Neidhardt (12)
K166(pFN97)	<i>recA srl</i> derivative of K165; plasmid is <i>htpR</i> ⁺	F. Neidhardt (45)
B178	W3110 <i>galE</i>	F. Neidhardt (45)
OF218	B178 <i>recA56 srl::Tn10</i>	C. Georgopoulos
Plasmids (all are Amp ^r)		
pBR322		(8)
pOF12	<i>groES</i> ⁺ <i>groEL</i> ⁺ insert into pBR322	C. Georgopoulos (19)
pOF13	<i>groES313 groEL</i> ⁺ insert into pBR322	C. Georgopoulos (19)
pOF14	<i>groES314 groEL</i> ⁺ insert into pBR322	C. Georgopoulos (19)
pOF15	<i>groES315 groEL</i> ⁺ insert into pBR322	C. Georgopoulos (19)
pS4	<i>groES</i> ⁺ <i>groEL del4</i> insert into pBR325	C. Georgopoulos (19)
pOF39	<i>groES</i> ⁺ <i>groEL</i> ⁺ insert into pBR325	C. Georgopoulos (19)

(10⁸) were as follows: SC122, 6.3 and 6.5; K165, 5.4 and 6.4; K166 (pFN97), 3.8 and 4.0. *E. coli* B grown in parallel gave values of 3.5×10^8 and 4.7×10^8 CFU/ml.

Growth conditions, media, and chemicals. All temperatures above 40°C were read on thermometers calibrated against a thermometer certified by the National Bureau of Standards to be accurate within 0.3°C. Unless noted otherwise, bacteria for phage infections were grown at 30°C in GCA (glycerol-Casamino Acids) medium (21), by inoculating with 0.01 volume of a stationary-phase culture and chilling when the A₄₉₀ reached 0.13 (1-mm light path). This yielded a cell concentration of around 4×10^8 cells per ml, based on colony-forming ability. For the experiments in Fig. 2 through 6, the cells were diluted in half with fresh GCA medium before they were warmed for the experiments; this dilution increased phage production per adapted cell by 30 to 40%. For *E. coli* SC122, K165, and K166 (pFN97), all media were supplemented with tryptophan (50 µg/ml) because they contain a *trp am* mutation; even though this *am* mutation is suppressed at 30°C, tryptophan generally improves growth rates at 30°C. M9C medium has been described previously (9). Preparation of concentrated T4 stocks and plating media and assay procedures for viable T4 and bacteria are as described by Hercules et al. (30); T2 and T6 were handled the same way. Plating of all phage was done on *E. coli* B except for T4 *am* mutants, which were plated on *E. coli* CR63, which contains *supD*. All infections were done at a multiplicity of infection of 5 to 9. All T7 dilutions for plating were made in Difco nutrient broth containing 0.59 M NaCl for phage stability (66). Concentrated stocks of T7 were prepared by infecting log-phase *E. coli* B in GCA medium at 37°C and a multiplicity of infection of 4. After about 1 h (lysis complete), CHCl₃ and NaCl (final concentration, 0.5 M) were added. This lysate was chilled and centrifuged at 4°C at $5,000 \times g$ for 15 min. The supernatant was centrifuged at $25,000 \times g$ for 2 h after 10 to 20 µg of sterile Celite (diatomaceous silicon dioxide; Johns Manville) was added to keep the T7 pellet from resuspending prematurely; the T7 pellet was suspended overnight at 4°C by overlaying it with Difco nutrient broth containing 0.59 M NaCl. The Celite and residual debris were removed by sedimentation at $5,000 \times g$ for 15 min.

The ¹⁴C-amino acid mixture (CFB.104), L-[methyl-¹⁴C]tryptophan (CFA.289), and [methyl-³H]thymidine (TRK.418) were from Amersham Corp. [2-³H]uracil (NET-333) was from New England Nuclear Corp. Carbenicillin was from Sigma Chemical Co.

Radioactive labeling and electrophoresis of *E. coli* proteins.

For the experiments in Fig. 1, M9C medium supplemented with methionine and tryptophan (100 µg/ml each) was inoculated at 30°C with 0.01 volume of an overnight culture in the same medium; methionine is required by wild-type *E. coli* at high temperature (56), whereas all bacteria used for the experiment in Fig. 1 (except *E. coli* B) require L-tryptophan at high temperature (Table 1). The cultures were chilled when a concentration of about 4×10^8 cells per ml was reached (3 to 5 h). Portions of 1 ml in 10-ml Erlenmeyer flasks were placed on a shaker bath at 30 or 42.8°C. At 0 min (at 30 and 42.8°C) and 3 or 6 min later (42.8°C), 25 µl of ¹⁴C-amino acid mixture was added to a final concentration of 1 µCi/ml (7 µg/ml). After 6 min (30°C) or 3 min (42.8°C) of labeling, 1 ml of cold 20% trichloroacetic acid was added, and the cells were harvested by centrifugation. Cells were washed and dissolved in buffer containing sodium dodecyl sulfate (SDS) (9). It is important to use 15 to 20% trichloroacetic acid in the determination of acid-insoluble radioactivity in such samples, because SDS causes incomplete precipitation of protein at lower levels of trichloroacetic acid (9). These samples were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) at 10°C on a 10 to 16% polyacrylamide gradient gel (18 cm long) with a stack of 4% polyacrylamide, essentially as described by Cardillo et al. (9), except that the mercaptoethanol concentration in the sample buffer was increased fivefold to 1.5%, and the stacking gel was at pH 6.8 instead of 8.8. The gel was dried and exposed to Kodak SB-5 film for 6 days.

For the experiments in Fig. 6, the gel procedures were similar, except that the separating gel was an acrylamide gradient of 8 to 16% in a 5 to 20% sucrose gradient.

RNA synthesis. One portion of an *E. coli* B culture (adapted) was allowed to grow for 8 min at 43.8°C before infection at a multiplicity of infection of 7. Another portion (unadapted) was placed at 30°C for 3 min and then infected; 5 min later the culture was transferred to 43.8°C. At various times starting at 4 min after infection (adapted cells) or at 4 min after transfer of the infected cells from 30 to 43.8°C (unadapted cells), 0.2 ml of culture was transferred to a 13-by-100-mm test tube (already at 43.8°C in the shaking water bath) that contained 10 µl of [5-³H]uracil such that the final concentration was 2 µCi/ml (0.25 µg/ml). After 4.5 min, 10 µl of uracil (2 mg/ml) was mixed in, and 75 µl of the contents of the tube was delivered to a cellulose filter disk (Whatman 3MM), which was immediately put into cold 10% trichloroacetic acid. The filters were washed further, and radioactiv-

ity was determined as described by Kutter and Wiberg (37). Control experiments showed that this level of uracil was not limiting under these conditions. Incorporation of exogenous uracil into T4 RNA is a reliable measure of T4 transcription (17).

DNA synthesis. Adapted and unadapted *E. coli* B were prepared as for RNA synthesis. Tryptophan (6 µg/ml, final concentration) was added to both cultures before they were warmed to temperature. After 5 min of growth of the infected cells at 44.0°C (adapted or unadapted), [methyl-³H]thymidine (specific activity, 42 Ci/mmol) and *d*-adenosine were added (37); the final concentrations were 1.5 µCi/ml and 250 µg/ml, respectively. Portions of 75 µl were sampled at intervals onto cellulose filter disks and processed as for RNA synthesis. Control experiments showed that this level of thymidine did not limit incorporation under these conditions; the rate of incorporation of thymidine into the infected cells can be adjusted at will, since it is inversely related to the concentration of *d*-adenosine (unpublished experiments).

Radioactive labeling and electrophoresis of T4 proteins. Adapted and unadapted *E. coli* B were prepared as for RNA synthesis. For the adapted set, unlabeled tryptophan (final concentration, 3 µg/ml) was added before the cells were warmed to 44.0°C. For the unadapted set, the tryptophan was added after the 3-min growth period at 30°C (just before infection). At 14 min after infection (adapted), or 14 min after shifting the unadapted infected cells from 30 to 44.0°C, [¹⁴C]tryptophan (final concentration, 1 µCi/ml) was added, bringing the total concentration of added tryptophan to 6 µg/ml. Control experiments showed that this level of tryptophan did not limit incorporation under these conditions. Tryptophan can be used to label in GCA medium (74) because Casamino Acids, produced by acid hydrolysis of casein, contain no tryptophan. At 45 min, unlabeled tryptophan (final additional concentration, 118 µg/ml) was added as a chase. Five minutes later, 50-µl samples were placed on cellulose filters and processed for total incorporation. The remaining cells were processed and subjected to SDS-PAGE essentially as described above, except that the separating gel was a 7 to 12% gradient of acrylamide in a 0 to 16% gradient of sucrose and was only 10 cm long.

Micrococcal nuclease assay. Portions (1 ml) of the same infected radioactive cells produced for Fig. 3 were transferred at 51 min to a test tube containing 0.2 ml of CHCl₃ and 0.1 ml of 0.03 M CaCl₂ at 37°C. After 8 min, 10 µl of micrococcal nuclease (Cooper Biomedical) was added to a final concentration of 20 µg/ml (30). Samples (75 µl) were transferred at various times to cellulose filter disks and processed as for RNA synthesis.

Effect of GroES- and GroEL-overproducing plasmids on T4 production. Cultures of *E. coli* OF218 carrying the various plasmids (Table 1) were grown at 30°C in GCA medium containing carbenicillin (50 µg/ml) to an A₄₉₀ of 0.040 (1-mm light path), to give a concentration of about 6 × 10⁷ cells per ml. Primarily to remove residual carbenicillin, the cultures were then chilled, and the cells were sedimented at 4,000 × *g* for 10 min and suspended in 0.5 volume of fresh, cold GCA containing tryptophan at 10 µg/ml. Adapted and unadapted cells were then prepared and infected exactly as for the experiments in Table 2, except that the high temperature was 43.7°C. Turbidity measurements at the start and end of the infections indicated that no lysis had occurred by harvest time. Portions of the uninfected, unadapted cells were sampled for examination of GroES and GroEL levels by SDS-PAGE. OF218(pOF39) adsorbed T4 much more poorly (al-

TABLE 2. Effect of adaptation of *E. coli* B to high temperature on production of phages

Treatment	Phage	PFU/CFU ^a	Final temp (°C)	Length of infection (min)
Low temp	T2 ^b	125	30	90
	T4 ^{b,c}	135	30	90
	T6 ^b	229	30	90
	T7 ^d	40	30	26
Unadapted cells ^e	T2 ^b	5.0	42.8	50 ^f
	T4 ^{b,c}	0.7	44	50 ^f
	T6 ^b	19	44	50 ^f
	T7 ^d	35	44	23 ^f
Adapted cells ^g	T2 ^b	75	42.8	45
	T4 ^{b,c}	187	44	50
	T6 ^b	156	44	50
	T7 ^d	155	44	22

^a Ratios of PFU/CFU for adapted cells to those with unadapted cells were as follows: 15 for T2, 267 for T4, 8.2 for T6, and 4.4 for T7.

^b No lysis was evident at harvest time, as judged by optical density.

^c Uninfected cells were grown to half the usual optical density.

^d Cells at 44°C had lysed by the time of phage harvest; however, the spontaneously released phage remained viable for at least another 20 min of incubation, so no loss of phage to debris was evident.

^e Infected at 30°C and shifted to high temperature 5 min (3 min for T7) later.

^f Includes the period at 30°C.

^g Grown at high temperature for 8 min before infection.

though adequately) than the other plasmid-containing strains; we have no explanation, but we suspect it has fewer adsorption sites. Plating the bacterial cultures with and without ampicillin (50 µg/ml) indicated that 100% (±10%) of the cells in each strain contained the plasmid.

RESULTS

Effect of heat shock on phage production. When *E. coli* B was grown at 30°C for many generations, infected with T2, T4, T6, or T7 phage for 3 to 5 min (thus unadapted to high temperature), and then shifted to high temperature, low to negligible levels of phage were produced (Table 2). However, when the bacteria were allowed 8 min of growth at the high temperature before infection (adapted), production of all four phage increased from 4.4-fold for T7, to 267-fold for T4 (Table 2). The data for phage production at 30°C are shown only for perspective; the only important comparison is that between adapted and unadapted cells. After infection by T2, T4, or T6, the *htpR* regulon of the host cannot be expressed, since T-even phage completely block expression of host genes within a few minutes of infection (64, 75). T7 is much slower to block expression of host genes (65); this is discussed below. In experiments not shown, T4 production at 44°C stopped at about 70 min after infection of adapted *E. coli* B, whereas phage production in the unadapted cells never rose above the low level seen at 50 min, even at 110 min after such infected cells were shifted to 44°C from 30°C; increasing the concentration of tryptophan from 6 to 30 µg/ml in the cultures did not alter these results. The use of tryptophan is explained below. Based on turbidimetry at 110 min after infection, the unadapted cells showed no lysis, and the adapted cells showed no more than 25% lysis.

Two other strains of *E. coli*, CR63 (a K-12 strain) and a different wild-type *E. coli* B (originally from Gunther Stent, *E. coli* Genetic Stock Center strain CGSC 2507), supported an equally dramatic improvement in T4 production upon

TABLE 3. Effect of *htpR* gene on T2 phage production^a

Treatment ^b	<i>E. coli</i> strain	Expt	Phage yield (10 ⁹ /ml)	% Un-adsorbed phage ^c	% Uninfected bacteria ^c
Low temp	SC122	1	43	31	11
		2	45	32	14
	K165	1	62	33	3
		2	26	17	6
	K166(pFN97)	1	42	32	8
		2	31	20	6
Unadapted cells	SC122	1	3.6		
		2	1.5		
	K165	1	1.9		
		2	1.1		
	K166(pFN97)	1	12		
		2	13		
Adapted cells	SC122	1	24	9	23
		2	32	1	18
	K165	1	2.1	2	1.5
		2	1.2	2	1
	K166(pFN97)	1	23	4	1
		2	32	2	1

^a Ratios of phage yield of adapted cells to that of unadapted cells were as follows (experiments 1 and 2, respectively): for SC122, 6.7 and 21; for K165, 1.1 and 1.1; for K166(pFN97), 1.9 and 2.5.

^b For the low temperature treatment, bacteria were held at 30°C for 3 min and infected at 30°C for 90 min. Unadapted bacteria were held at 30°C for 3 min, infected at 30°C for 5 min, and shifted to 42.8°C for 45 min. Adapted bacteria were grown at 42.8°C for 8 min and infected at 42.8°C for 45 min.

^c Determined at 5 min postinfection for low-temperature-treated bacteria and after 5.5 min at 42.8°C for adapted bacteria.

adaptation to 44 or 45°C (data not shown); in contrast to the *E. coli* B in Table 1, CGSC 2507 does not sport filaments.

Since the physiological effects of heat shock are so dramatic, resulting in a rapid and marked increase in synthesis of at least 17 specific *E. coli* proteins (46), we suspected that it is the expression of the *htpR* regulon that rescues phage production at high temperature. This was shown (Table 3) by use of a host containing an *htpRam* mutation coupled to a temperature-sensitive suppressor; at high temperature the *htpR* gene is not expressed functionally. *E. coli* SC122 (*htpR*⁺) supported a heat shock effect whereby its adaptation to 42.8°C for 8 min before infection increased T2 production 6.7- and 21-fold in two separate experiments (Table 3). In contrast, K165 [*htpRam*, *supC* (*ts*)] did not support a significant increase in T2 production under the same conditions (Table 3). Insertion of an *htpR*⁺ plasmid into K166 (a *recA* derivative of K165) completely restored phage production in the cell first adapted to 42.8°C (Table 3).

It is interesting that T2 production in unadapted cells at 42.8°C was 6 to 12 times higher in K166(pFN97) than in K165, a nearly isogenic but plasmid-free strain (Table 3). Since this plasmid contains the *htpR*⁺ gene, a reasonable explanation of the higher phage production is that the plasmid-borne *htpR*⁺ gene is expressed at 30°C, whereas the *htpR*⁺ gene on the host genome is expressed very little. Figure 1 supports this interpretation; heat shock protein bands b, c, and d are considerably overproduced at 30°C in K166(pFN97) relative to K165. Band d is undoubtedly the GroEL protein (see below). This overproduction of heat shock proteins at 30°C in K166(pFN97) thus presumably explains the low ratio of adapted to unadapted phage production seen in Table 3.

We have carried out experiments with T4 and T6 that are

comparable to the T2 experiments of Table 3, with similar results; i.e., significant phage production at high temperature required expression of the *htpR* gene (data not shown).

In an important control experiment (Fig. 1), the four uninfected bacterial strains exhibited the heat shock response expected from their genotypes. Strains B, SC122, and K166(pFN97) (all *htpR*⁺) but not K165 (*htpR*) exhibited a marked increase in the labeling of the seven proteins marked a through g, after the shift to 42.8°C. A comparison of this gel with a similar gel (78) argues that proteins a, b, c, and d have molecular weights of about 87,000, 76,000, 73,000, and 64,000, respectively, and that protein d is GroEL. In contrast, strain K165 (*htpRam*, *supCts*) failed to exhibit these increases in the rates of labeling the seven proteins.

RNA synthesis. We then began an effort to learn which metabolic processes are deficient in the T4-infected, unadapted cells at high temperature. RNA synthesis was measured as incorporation of [5-³H]uracil into acid-insoluble material at 43.8°C; with [5-³H]uracil, no label enters DNA because the 5-³H atom is lost upon conversion of uracil to thymine or to hydroxymethylcytosine and because T-even phage contain hydroxymethylcytosine to the absolute exclusion of cytosine (38, 76). It appeared at first that the adapted cells supported much less T4 RNA synthesis than did the unadapted cells (Fig. 2A). However, since we had expected that, if anything, the unadapted cells would exhibit the lower RNA synthesis, we suspected an artifact; the following arguments supported this suspicion.

Edlin and Neuhaud (16) showed that uninfected *E. coli* are unable to take up [¹⁴C]uracil into nucleotides under stringent conditions (*rel*⁺ and amino acid starvation), whereas uptake does occur in *rel*, amino acid-starved cells; adenine uptake was much less affected. Donini and Edlin (14) and Fast and Sköld (18) observed a severe restriction of U uptake into

FIG. 1. Induction of heat shock proteins in various uninfected *E. coli* strains. Shown are gel patterns after SDS-PAGE of ¹⁴C-amino acid-labeled proteins. Label was present for 6 min at 30°C and in three successive 3-min pulses after transferring the cells to 42.8°C. Samples of 16,000 cpm were applied to each gel. Bands f and g did not completely separate here but did so on other gels with the same samples.

T4-infected cells under stringent conditions by using amino acid starvation to induce stringency; under the same conditions, adenine uptake and incorporation into RNA were excellent. Furthermore, stringent conditions have a negligible effect on T4 RNA synthesis (41). Since we had not deliberately imposed amino acid starvation on our infected-cell system (with wild-type *E. coli* B), we did not expect a deficiency in uracil uptake. A probable explanation was suggested by the work of Ron and Davis (56), who found that *E. coli* B and several other *E. coli* strains grow progressively slower as the temperature is raised above 40°C in minimal medium and that methionine blocks this effect. This suggested to us that high temperature imposed a partial amino acid starvation on the adapted, T4-infected *E. coli* B, triggering a stringent response that caused some loss of the ability to take up uracil. In support of this, we found that tryptophan corrected the deficiency (Fig. 2B); more methionine (which is already present in GCA medium) had no effect. The gradual decrease in rate of U incorporation with time after infection (Fig. 2B) is virtually the same as that seen at 37°C (data not shown) and has been seen before in normal T4 infections (63). We conclude from the data in Fig.

FIG. 2. Rate of RNA synthesis as a function of time after T4 infection of *E. coli* B at 43.8°C: effect of adaptation of the host to 43.8°C before infection and effect of tryptophan. Pulses (4.5 min) of [^3H]uracil were begun at the times shown. Shortly before the cultures of part B were warmed to temperature, tryptophan was added to give 3 $\mu\text{g}/\text{ml}$. Phage production per cell at 50 min was (A) 4.1 and 118 and (B) 3.8 and 161 in unadapted and adapted cells, respectively.

FIG. 3. DNA synthesis at 44.0°C after infection of *E. coli* B by T4 $^+$: effect of adaptation of host to 44.0°C before infection. Cumulative incorporation of [$\text{methyl-}^3\text{H}$]thymidine was begun at 5 min. Tryptophan (final concentration, 6 $\mu\text{g}/\text{ml}$) was added to both cultures at the start of the experiment. Phage production per cell by T4 $^+$ at 50 min was 310 (adapted) and 7.8 (unadapted).

2B that the rate of T4 RNA synthesis is not affected significantly when the uninfected cells are not allowed to adapt to the high temperature.

DNA synthesis. Continuous incorporation of [^3H]thymidine into DNA was measured at 44.0°C after infection by T4 $^+$ (Fig. 3). Net DNA synthesis by T4 $^+$ (unadapted) by 50 min was 69% of that by T4 $^+$ (adapted). We conclude that T4 $^+$ DNA synthesis is reduced only about 30% by failure to adapt the uninfected cells to high temperature.

Protein synthesis. To measure the effect of adaptation on both the rate of T4 protein synthesis and the amounts of specific T4 proteins made, T4-infected cells were labeled in *E. coli* B with [^{14}C]tryptophan, and the proteins were separated by SDS-PAGE (Fig. 4); 63% as much [^{14}C]tryptophan was incorporated into phage protein in unadapted cells as in adapted ones between 14 and 45 min of growth of the infected cells at 44.0°C. This value was about 80% in other experiments at 43.8°C, in which label was present from the start of infection (data not shown). Thus, an average of 28% of the nearly total loss of phage production in unadapted cells can be accounted for by a decrease in overall protein synthesis. The separated proteins are displayed in Fig. 4. As controls, IPIII $^-$, 23 $^-$, 24 $^-$, and 23 $^-$ 24 $^-$ infections were included to help identify the respective proteins and their processed (*) forms. Proteins gp24 and gp24* migrate to about the same position as gp23* in such gels (71). However, neither gp24 nor gp24* was evident on the gel; this probably reflects the relative abundance of gp23* and gp24* in the mature phage particle: 1,000 and 65 molecules, respectively (6). The failure to detect gp24 or gp24* in the 23 $^-$ extracts is almost certainly not due to a polarity effect of the 23 $^-$ amber mutation on gene 24, despite the position of the mutation (*amH11*) near the 5' end of the gene (51); this is because gene 23 transcripts apparently end before gene 24 (51). With two

exceptions, the pattern with adapted $T4^+$ was remarkably similar to that with unadapted $T4^+$; lack of adaptation caused a significant reduction in processing of gp23 to gp23*, and of IPIII to IPIII*, with $T4^+$. gp23* contains only 75% of the ^{14}C that the same number of molecules of gp23 contains, since gp23* contains three tryptophan molecules, whereas gp23 contains four (51). In contrast, all of the tryptophan in IPIII remains in IPIII* (32).

Visual inspection of several different exposures of the autoradiogram of Fig. 4 indicated that about the same total amount of gp23 (the lower of two close bands) plus gp23* was made whether the cells were adapted or not; the same was true of IPIII plus IPIII*. However, it is the processed form of gp23 that is required for viable phage particles, and the level of gp23* was significantly reduced in the unadapted, $T4^+$ -infected cells relative to the adapted, $T4^+$ -infected cells. In contrast, neither IPIII nor IPIII* is absolutely required for viability (5). We observed that the IPIII^{am} mutant produced 27% as many viable phage particles at 50 min in adapted cells as did $T4^+$ (Fig. 4). IPIII⁻ mutations reduce the processing of gp23 at 37°C (5); processing of head-related proteins appears to be cooperative (6). In agreement with this, Fig. 4 shows reduced processing of gp23 at 44°C in IPIII⁻ amber mutant infections. Further-

FIG. 4. Patterns after SDS-PAGE of [^{14}C]tryptophan-labeled T4 proteins: processing of T4 gene 23 protein and IPIII in adapted (AD) versus unadapted (UN) cells at 44.0°C. Label was added after 14 min of growth of the infected cells at 44.0°C and chased with unlabeled tryptophan for 5 min at 45 min. At 50 min the cells were harvested and prepared for electrophoresis as described in Materials and Methods. Phage production per cell was as follows: at 25 min, unadapted, 6 $T4^+$, 4.2 IPIII⁻; at 50 min, unadapted, 16 $T4^+$, 5.9 IPIII⁻; at 25 min, adapted, 130 $T4^+$, 52 IPIII⁻; at 50 min, adapted, 319 $T4^+$, 86 IPIII⁻. The 23⁻ and 24⁻ mutations completely block phage production. A sample of 5,500 cpm was applied to each lane. Acid-insoluble radioactivity (cpm) in 50- μ l samples of whole cells at 50 min was as follows. Unadapted: $T4^+$, 3,970; 23⁻, 4,130; 24⁻, 4,520; 23⁻ 24⁻, 4,420; IPIII⁻, 4,420. Adapted: $T4^+$, 6,320; 23⁻, 5,150; 24⁻, 5,740; 23⁻ 24⁻, 5,060; IPIII⁻, 6,050. Thus, unadapted $T4^+$ incorporated 63% as much label as adapted $T4^+$, which compares well with the value of 78% determined in another, comparable experiment at 50 min at 43.8°C. Gene 34 protein (gp34) and gp37 are the proximal and distal tail fibers, respectively, and gp7 is a component of the tail baseplate.

FIG. 5. Micrococcal nuclease sensitivity of T4 DNA produced in *E. coli* B at 44.0°C: effect of adaptation of host to 44.0°C. Acid-precipitable ^{14}C is plotted. The 0-min values are the 50-min values of Fig. 3, except that they have been divided by 1.1 because of the $CaCl_2$ addition and divided by 0.75, the ratio of [3H]DNA counting efficiency in whole cells to that in lysed cells (unpublished data); apparently the washed and desiccated whole cells provide a small amount of shielding. Survival of $T4^+$ in the adapted lysate at 1,260 min was 76%.

more, significantly less IPIII* is produced in the unadapted cells than in the adapted ones (Fig. 4).

Is the DNA packaged? Since processing of gp23 to gp23* and of IPIII to IPIII* was considerable in the unadapted cells (Fig. 4) and thus did not provide a complete explanation for the near absence of phage production, we asked how much of the DNA produced (Fig. 3) was encapsidated. Cells labeled with [3H]thymidine for Fig. 3 were lysed and treated with micrococcal nuclease (Fig. 5). About 50% of the $T4^+$ DNA made in adapted cells at 44°C remained resistant to the nuclease for at least 21 h at 37°C, arguing that this amount was packaged securely into heads. In contrast, only 4.6% of the $T4^+$ DNA made in the unadapted cells at 44°C survived nuclease treatment. Since the unadapted $T4^+$ infection yielded 2.5% as many viable phage as did the adapted $T4^+$ infection, about half of the nuclease-resistant DNA in the unadapted $T4^+$ infection is accounted for by complete phage particles. Thus, a sufficient general explanation for the nearly total loss of phage production in unadapted cells is a failure to package the DNA.

Effect of GroEL- and GroES-overproducing plasmids. One of the *E. coli* heat-shock proteins, GroEL, is absolutely required for T-even phage production. The protein products of gene *groEL* and T4 genes 31 and 23 (the major head structural protein) appear to interact at the inner membrane of the host to form a complex necessary for maturation of T4 heads (4, 24); e.g., a mutation in *groEL* or in T4 gene 31 completely blocks proteolytic processing of T4 gp23 (24), thereby blocking T4 phage production. To test whether the

TABLE 4. Effect of GroEL- and GroES-overproducing plasmids on T4 production in unadapted *E. coli* OF218 cells at 43.7°C

Plasmid	Expt	Phage per cell		Unadapted/ adapted ratio	Avg ratio
		Unadapted	Adapted		
pBR322 (<i>groES groEL</i>)	1	27	251	0.105	0.107
	2	29	269	0.108	
pOF12 (<i>groES⁺ groEL⁺</i>)	1	86	193	0.45	0.46
	2	99	216	0.46	
pOF13 (<i>groES groEL⁺</i>)	1	37	78	0.47	0.37
	2	23	85	0.27	
pOF14 (<i>groES groEL⁺</i>)	1	57	118	0.48	0.44
	2	31	77	0.40	
pOF15 (<i>groES groEL⁺</i>)	1	76	173	0.44	0.35
	2	39	148	0.26	
pS4 (<i>groES⁺ groEL</i>)	1	2.8	111	0.025	0.022
	2	2.2	124	0.018	
pOF39 (<i>groES⁺ groEL⁺</i>)	1	67	135	0.50	0.44
	2	76	205	0.37	

marked deficiency in T4 production in cells unadapted to high temperature is due to a deficiency in GroEL, bacterial strains containing plasmids that overproduce GroEL (19) were studied. As usual, phage production at high temperature was compared between cells unadapted to high temperature before infection and those adapted for 8 min. Cells containing plasmids pOF12, pOF13, pOF14, pOF15, and pOF39, all of which contain the *groE⁺* gene, supported production of 37 to 46% as many phage in unadapted cells as in adapted cells (Table 4). In contrast, cells containing a plasmid (pBR322) without a *groEL* insert or containing a nonfunctional portion of *groEL* (pS4) supported production of only 11 and 2.2% as many phage, respectively, in unadapted cells as in adapted cells. Thus, roughly half of the deficiency in phage production at high temperature in unadapted cells is due to a deficiency in GroEL. In three of the *groEL⁺* plasmids, *groES* is mutant (a two-base frameshift in pOF13 and three contiguous base-pairs deleted in pOF14 and pOF15), yet the same percent rescue was seen as when the plasmid *groES* was the wild type (Table 4). Thus GroES appears to play no role in the heat shock effect on T4 production; this agrees with observations that all *groES* mutants examined are permissive for T4 production (68).

As an important control, the levels of GroEL and GroES in the plasmid-containing strains were examined (Fig. 6). GroEL was greatly overproduced whenever the plasmid was *groEL⁺* and was barely detectable when the plasmid lacked *groEL* (pBR322) or when most of *groEL* was deleted (pS4). Thus the considerable rescue of T4 production by certain plasmids (Table 4) correlates with overproduction of GroEL.

Production of GroES by the cell harboring pBR322 was undetectable. However, the three *groES⁺* plasmids did produce GroES, but in disparate amounts (pOF39 > pOF12 > pS4). This variation may reflect disparity in plasmid copy number (not tested); this interpretation is supported by a corresponding variation in production of GroEL by the two of these three plasmids (pOF12 and pOF39) that are *groEL⁺* (Fig. 6 and data not shown). Note that the mutant GroES protein from pOF15 had higher-than-normal mobility; this confirms the observation of Fayet et al. (19), who examined stationary-phase cells of a different host grown at 42°C.

Plasmid pS4 produced much less GroES than the other *groES⁺* plasmids (Fig. 6); this apparently is not due to loss of *groES⁺* from the plasmid by recombination with the host gene, since the UV sensitivity of OF218(pS4) was as high as that of the other strains in Table 4 (data not shown), all of which are *recA* and thus unable to support such recombination.

Heat-shock effect on T7 does not involve the *htpR* gene. T7 production in *E. coli* B exhibited a 4.4-fold increase at 44°C when the cells were first adapted to growth at 44°C (Table 2). To learn whether this increase is due to heat-induced expression of the *htpR* gene, *E. coli* SC122 and K165 were employed again. After testing of several different tempera-

FIG. 6. SDS-PAGE test of overproduction of GroES and GroEL from plasmids; the gel was stained with Coomassie brilliant blue G-250. The cells were uninfected and grown at 30°C for experiment 1 of Table 4. The same number of viable cell equivalents were run in each lane. The plasmids (Tables 1 and 4), all in *E. coli* OF218, are as follows (lanes): 1, pBR322; 2, pOF12; 3, pOF13; 4, pOF14; 5, pOF15; 6, pS4; 7, pOF39.

tures, 42.7°C was found to provide the largest ratio in T7 production between adapted and unadapted *E. coli* SC122 (*htpR*⁺), given the requirement for a reasonable yield of phage in the adapted cell. This ratio was 1.8, i.e., half that seen in *E. coli* B at 44°C (Table 2). Most importantly, T7 production on K165 (*htpR* at high temperature) was essentially the same as on SC122 (*htpR*⁺ at high temperature) under all conditions, such that adaptation of K165 to 42.7°C increased T7 production 2.3-fold over that in unadapted cells. Phage yields (10⁹ per ml) on *E. coli* SC122 and K165, respectively, were as follows: with low-temperature treatment (30°C for 3 min, infection at 30°C for 70 min), 24 and 27; for unadapted cells (30°C for 3 min, infection at 30°C for 3 min, shift to 42.7°C for 23 min), 13 and 8; for adapted bacteria (growth at 42.7°C for 8 min, infection at 42.7°C for 23 min), 23 and 18. Thus the heat shock-induced increase in T7 production does not appear to be due to activation of the *htpR* gene; a control experiment was performed in parallel on the same bacterial cultures, showing that a typically large heat shock response was shown by T2 and that it was controlled by the *htpR* gene (data not shown).

In contrast to T4, T7 is slow to shut off synthesis of host proteins, taking up to 8 min at 30°C in M9 medium (65); we have found that this shutoff takes about 10 min in *E. coli* B at 30°C in GCA medium (data not shown). Since heat shock proteins other than those controlled by *htpR* exist (44), it seemed possible that these other heat shock genes might be expressed for a while upon shifting infected *E. coli* B to 44°C, when only 3 min of infection at 30°C had been allowed. If this is true, a reasonable prediction is that longer growth of infected cells at 30°C before temperature shiftup would reduce the potential for synthesis of these heat shock proteins at 44°C, thus reducing T7 phage production at 44°C. However, phage production at 44°C was not affected significantly by delaying the shiftup as long as 10 min (Fig. 7), thus failing to support the model; further delay gradually increased phage production about fourfold. As a control, phage production was followed also at 30°C; very few phage had been made before the shiftups shown (Fig. 7). An explanation for the late rise in T7 production at 44°C (Fig. 7) might be that certain events must occur after infection at 30°C before phage production becomes maximally resistant to heat shock; these events might be (i) complete shutoff of host protein synthesis or (ii) expression of certain T7 genes.

DISCUSSION

At least part of the increase in T4 DNA and protein synthesis (Fig. 3 and 4) after 8 min of adaptation of uninfected cells to high temperature must be due to increased amounts of basic cellular components: ribosomes, enzymes, membrane, etc. However, this unavoidable background fades to insignificance against the dramatic, specific changes embodied in the heat shock response. Thus, for an explanation of the enormously higher T4 phage production in adapted cells, we have looked to the heat shock proteins themselves. Among the 17 *E. coli* heat shock proteins so far recognized, the genes encoding 8 have been identified; these are *dnaJ*, *dnaK*, *groEL* (*mopA*), *groES* (*mopB*), *lysU*, *rpoD*, *lon* (52), and *grpE* (1). Gene *groEL* was a strong candidate from the beginning, as it is required for production of T2, T4, and T6, through its obligatory role in processing T4 head proteins. If the level of gp23* were the only relevant factor we could say that the partial deficiency in processing gp23 (Fig. 4) can explain only a small decrease in T4 production at 44°C in unadapted cells, whereas the observed decrease was

FIG. 7. T7 production at 44.0°C: effect of duration of prior infection at 30°C. *E. coli* B was grown at 30°C and infected at a multiplicity of 7. In two separate experiments (○, ●), cells were shifted to 44.0°C at the times shown and sampled 25 min after the shift. As a control in the same two experiments, portions of infected cells were left at 30°C and sampled at the times shown (□, ■). Values for phage per cell at 25 min after infection of cells adapted for 8 min at 44.0°C and then infected at 44.0°C were 122 (●, ■) and 70 (○, □).

95% (Fig. 4). However, it is conceivable that at least a few of the 1,000 molecules of gp23 remain unprocessed in each of 95% of the heads produced in unadapted cells and that such hybrid heads are not functional. To accommodate the nuclease results of Fig. 5, such hybrid heads would have to be unable to package DNA properly.

An earlier study from this laboratory examined the effects of high temperature on T4 head assembly (74). Since the experimental conditions differed in a number of respects from those used here, an exact comparison is not possible. Nonetheless, SDS-PAGE showed that less processing of gp23 to gp23* occurred in unadapted cells than in adapted cells at 44.8°C. Electron microscopy showed that, in unadapted cells in which two viable phage were produced per cell, elongated heads were the predominant head structure, followed by preheads, empty capsids, and DNA-filled heads without tails. It was also shown that gp24*, the mature head vertex protein, was greatly underproduced in unadapted cells; thus it was suggested that the amount of gp24* was inadequate to stabilize the end caps, resulting in excessive head elongation.

Rao and Black (55) recently showed that the T4 DNA packaging enzyme, which is a DNA-dependent ATPase and a sequence-nonspecific endonuclease, is another proteolytic product of gp23, i.e., gp23**. They estimated that 3 to 6 molecules of gp23** are in a prohead, compared with 1,000 molecules of gp23, and that gp23** is not found in mature phage particles. They also showed that formation of gp23** depends on head-protein processing. Thus it is reasonable to

expect that formation of gp23** is also reduced in unadapted cells at high temperature and that this reduction contributes to the virtual abolition of nuclease-stable DNA (Fig. 5); i.e., some otherwise mature heads may lack enough gp23** to cut the DNA, thus preventing closure of the DNA entrance vertex and leaving the almost-packaged DNA vulnerable to micrococcal nuclease. Thus, if processing of gp23 to gp23** were more sensitive to a deficiency in GroEL than processing of gp23 to 23*, this might explain completely the near-total susceptibility to micrococcal nuclease of the T4 DNA in unadapted cells.

The demonstration that *groEL*⁺ plasmids restore T4 production in unadapted cells to nearly 50% of that in adapted cells (Table 4) argues that a deficiency in *GroEL* in unadapted cells lacking these plasmids is responsible for about half the decrease in T4 production caused by lack of expression of the *htpR* regulon at high temperature. However, it may be that a deficiency in GroEL is the only cause of decreased T4 production, as follows. (i) It is clear from Fig. 6 that GroEL is present in considerable excess in the cells containing *groEL*⁺ plasmids; e.g., pOF39 (lane 7) produces at least fourfold as much GroEL as does pOF12 (lane 2), yet both restore phage production to the same value. (ii) The gel of Fig. 1 shows the rate of incorporation of ¹⁴C-labeled amino acids into GroEL (band d) during induction of the heat shock effect. On the stained version of this gel (not shown), comparison of the stain intensities of the GroEL bands with those of neighboring bands shows clearly that the amount of GroEL produced in those *E. coli* strains, in response to 9 min of heat shock, is at least 10 times lower than the lowest level induced by a *groEL*⁺ plasmid (Fig. 6, pOF12). The conditions for the experiment of Fig. 1, especially the temperature (42.8°C), were the same as for the T2 experiment of Table 2; thus the level of GroEL produced by pOF39 is at least 40 times that needed for rescue of T2 at high temperature. (iii) Thus we suggest the possibility that too much GroEL may interfere with T-even phage production and that this could explain the incomplete rescue of T4 production by the *groEL*⁺ plasmids.

An alternative explanation for the incomplete rescue by GroEL may be that one or more of the other *E. coli* heat shock proteins, except GroES, is also involved in the rescue; thus we briefly consider the others. (i) The *rpoD* gene encodes σ 70, the major sigma subunit of RNA polymerase. σ 70 plays a role in both early and late stages of T4 infection (82), but the details are incomplete (54). The HtpR protein (σ 32), which activates transcription at heat shock promoters, exhibits considerable amino acid sequence homology with σ 70 (27, 39). (ii) The *E. coli* DnaK and DnaJ functions are required for λ phage replication (83), but we are unaware of any evidence that they are required for T-even phage replication. (iii) The *lon* gene encodes a protease that apparently is involved in degrading abnormal proteins (3, 10, 25). T4 and T7 infections result in marked inhibition of host proteolytic activity against abnormal proteins (61, 62), and there is evidence that suggests that T4 infection inhibits the Lon protease: although the initial protease activity in a *lon*⁺ cell is higher than that in a *lon* cell (carrying a Tn10 insertion in the *lon* gene), the activities in both infections decrease to similar low values by 10 min after infection at 37°C (L. D. Simon, personal communication). (iv) The *lysU* gene encodes an isospecies of lysyl-tRNA synthetase that is only made to a significant extent under certain conditions, including high temperature (46). T4 is not known to produce its own lysyl-tRNA synthetase. However, Neidhardt et al. (46) have suggested that the LysU protein may serve a wider role

in the heat shock response through its presumptive synthesis of alarmones, which are various phosphorylated derivatives of adenosine (72), but it is unclear how such a role of the LysU protein might affect T4 production. (v) The *grpE* gene is necessary for growth of *E. coli* and for maintenance of *E. coli* DNA and RNA synthesis, at least at high temperature, and appears to be required for λ DNA replication at all temperatures (1, 58, 59). However, we are unaware of any evidence for a role of GrpE in T4 replication.

A highly precise route to learning the high-temperature role of any given heat shock protein, both (i) in increasing production of any virus and (ii) in maintaining the health of the uninfected host cell, would be the following. Given that (i) the heat shock-regulatory gene of the host (e.g., the *E. coli* *htpR* gene) encodes a sigma-like factor (e.g., σ 32 in *E. coli*) that activates transcription of a specific heat shock promoter serving the gene that encodes a given heat shock protein (27, 67), and given that (ii) the predominant sigma factor (σ 70) still functions at a different promoter in that same gene, one need only inactivate the particular heat shock promoter (by in vitro site-directed mutagenesis, e.g.). This approach offers the possibility of selectively blocking the heat-induced overexpression of one heat shock gene at a time without blocking the normal expression of that gene, e.g., from one or more σ 70-sensitive promoters. However, this approach will be complicated when applied to the *dnaK* and *rpoD* genes because the DnaK protein is responsible for the eventual inhibition of the heat shock response (69) and there appears to be functional interaction or competition between the RpoD protein (σ 70) and the HtpR protein (σ 32) (27, 29, 50, 77).

The fact that addition of tryptophan considerably improved uracil incorporation in T4-infected, adapted cells but not in T4-infected, unadapted cells (Fig. 2) argued that a partial tryptophan deficiency had developed during the 8 min of adaptation to 44°C and that this triggered a partial stringent response. Partial amino acid charging of a tRNA has been shown to cause a partial stringent response (7, 20, 22, 53). Perhaps the partial tryptophan deficiency indicated in Fig. 2 is explained by (i) an enzyme of the tryptophan synthetic pathway folding into an abnormal, less efficient conformation during its synthesis at 44°C or (ii) expression of the heat shock regulon somehow imposing a greater demand for tryptophan. Grossman et al. (28) showed that the stringent response induces expression of *E. coli* heat shock genes. This observation does not appear to bear on our stringency effect (Fig. 2A), because (i) the effect of any induction of heat shock genes by our stringency effect (Fig. 2A) should be masked by the simultaneous, deliberate induction of the heat shock genes caused by the 8 min of adaptation to high temperature and (ii) in Fig. 2B the tryptophan supplement apparently has prevented a stringent response.

An interesting evolutionary question is raised by our results: why did *E. coli* evolve an adaptive mechanism (one or more heat shock regulons) in such a way that it also benefited some of its predators (bacteriophage)? The answer may be that phage are obligate parasites and thus must also evolve to take best advantage of the vicissitudes of their hosts. Another question is, why does high temperature impose on T4 a greater need for GroEL protein? Perhaps at high temperature (i) GroEL is less stable or (ii) GroEL has a decreased affinity for the T4 head maturation complex on the membrane.

The apparent independence of the T7 heat shock response from the *htpR* gene invites several explanations. (i) As noted

in Results, perhaps heat shock proteins are involved that are not controlled by the *htpR* gene. (ii) Perhaps the response requires only minimal expression of *htpR*, and this expression may occur briefly during the shift from 30°C to high temperature before total inactivation of the temperature-sensitive suppressor in strain K165. Yura et al. (79) showed with presumably unsuppressed *htpR(am)* mutants that the *htpR* gene is not required for growth of *E. coli* below 34°C. Although they implied that the basal-level synthesis of GroE protein was not affected by the *htpR(am)* mutations at 30°C, it is possible that the synthesis of other *htpR*-controlled, heat shock proteins is affected. We have attempted to examine the T7 heat shock effect in these strains, but technical problems have interfered. (iii) Perhaps T7 infection itself activates the overexpression of one or more of the *E. coli* genes in the *htpR* regulon, as phage λ does (15, 36). Finally, if no heat shock proteins are involved in the 4.4-fold increase in T7 production on *E. coli* B caused by first adapting the cells to growth at 44°C for 8 min (Table 2), we suggest that heat-induced changes in membrane composition (13, 34, 57) may be responsible.

Some practical consequences of adapting host cells to high temperature before infection by the T-even and T7 phages include the following. (i) It raises the upper limit of useful temperatures for studies of phage physiology. (ii) Use of these higher temperatures should convert many leaky, temperature-sensitive (*ts*) phage mutants to nonleaky forms; i.e., the higher temperatures should totally inactivate some *ts* proteins not totally inactivated at previous temperature maxima.

Waghorne and Fuerst (73) showed that production of bacteriophage λ at high temperature requires expression of the *E. coli htpR* gene. The requirement was seen both for lytic growth and induction of lysogens. Host *htpR* mutations at high temperature caused defects in both head and tail formation. It was suggested that a deficiency in one or both of the *E. coli* GroE proteins contributes to the head defect, but that the *E. coli* DnaK protein is not limiting, even though it is required for λ DNA replication.

Various insect and animal viruses affect the rate of synthesis of certain heat shock or stress proteins (11, 23, 26, 33, 35, 40, 42, 43, 47–49, 60) and affect host survival after heat shock or stress (49); also, heat shock affects the expression of some viral genes (31, 80). Recently, Zerbini et al. (81) produced a marked increase in human cytomegalovirus production at 44°C by adapting the host cells to 44°C for 10 min before infection. However, to our knowledge, no reports have appeared except that of Waghorne and Fuerst (73) and our present results that demonstrate directly the involvement of the heat shock regulatory gene of a host (analogous to the *E. coli htpR* gene, for example) in increased production of viable viruses. It will be of considerable interest if activation of host heat shock (or other stress) regulons can be shown to increase the production of medically important viruses.

We do not plan to pursue further the findings of this report; we hope that others will.

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