Roles of the *Escherichia coli* Heat Shock Sigma Factor 32 in Early and Late Gene Expression of Bacteriophage T4

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We have analyzed early and late T4 gene expression at the levels of transcription and translation in *rpoH*⁺ (sigma 32⁺) and *rpoH* mutant cells infected under heat shock conditions. We found, as expected, that *Escherichia coli* cells must be adapted before infection to high temperature by the heat shock response to allow early T4 transcription, subsequent late gene expression, and progeny production at 42°C. Unexpectedly, we found in addition that when *rpoH* mutant (sigma 32 mutant) cells were shifted from 30 to 42°C 10 min after infection, late T4 genes were not expressed, even though DNA synthesis appeared to be normal.

We have investigated the effects of the *Escherichia coli* heat shock response on bacteriophage T4 development since this response has important consequences for design and interpretation of experiments at high temperatures (e.g., 42°C), a condition often used with temperature-sensitive mutants. In our early experiments on T4 DNA replication, using temperature-sensitive host mutants (13) or phage mutants (14) and their wild-type parents, we observed that host cells had to be adapted to 42°C, the restrictive temperature, before infection to obtain reproducible results (most data not shown). The discovery of the heat shock response suggested a possible reason for previous inconsistencies.

Transcription of T4 depends on *E. coli* core RNA polymerase which, together with various *E. coli* or phage-encoded transcription factors, recognizes different classes of phage promoters (2, 4). T4 early promoters require the major *E. coli* sigma factor, sigma 70 (17). The enhanced association of a minor sigma factor, sigma 32 (*E. coli* gene product [gp] *rpoH*), with RNA polymerase and the concomitant reduction in sigma 70 levels and activity after cells are shifted from 30 to 42°C (heat shock conditions) (19) is critical in the induction of *E. coli* heat shock genes (6, 7, 16, 25). The transcription of *rpoD* (sigma 70) from a heat shock promoter under these conditions is thought to be essential, among other factors, for the replenishment of sigma 70 and the resumption of global transcription (and therefore T4 transcription) after cells are adapted to higher temperatures (15, 21).

Since T4 infection turns off expression of all host genes (20), these considerations imply that sigma 70 is in low supply for early T4 transcription when wild-type or *rpoH* mutant host cells are shifted to a high temperature exactly at the time of infection. Thus, we expected that expression of early T4 genes, and consequently the entire T4 transcriptional program, might be defective under these conditions. Sufficient sigma 70 should be available for T4 transcription if *rpoH*⁺ cells have been adapted to higher temperatures before infection. In contrast, in hosts that are defective in the heat shock sigma factor (sigma 32), adaptation and sigma 70 replenishment cannot occur, regardless of the time elapsed after the heat shock. Thus, if the temperature is raised before infection, *rpoH*⁺ cells should allow T4 tran-

scription but *rpoH* mutant cells should not. Our first sets of results are consistent with these expectations.

Because late T4 promoters are recognized by a T4-encoded sigma factor, sigma gp55, and there is conflicting evidence whether they require host sigma factors (10, 26), we also tested the effects of the host *rpoH* mutation when cells were shifted from 30 to 42°C 10 min after infection, i.e., at a time when all the factors required for late gene expression, including gp55, should have been synthesized (17). We found that the heat shock sigma factor 32 is required for late transcription when infected cells are heat shocked at a time when T4 sigma gp55 has already been synthesized.

Expression of early T4 genes. Shifting the temperature at the time of infection drastically reduced T4 prereplicative and late proteins (Fig. 1), and consequently progeny production (Table 1), in both *rpoH*⁺ (*E. coli* SC122 and B strains were tested; only SC122 results are shown) and *rpoH* (K165) mutant host cells (Fig. 1, lanes 1 and 2). If *rpoH*⁺ cells were shifted to 42°C, grown at this temperature for 30 min (allowing heat shock induction of sigma 70), and then infected, T4 early proteins accumulated (Fig. 1, lane 5). In contrast, when *rpoH* mutant cells, which cannot synthesize sigma 70 in response to heat shock, were infected under these same conditions, all early T4 proteins were drastically reduced (Fig. 1, lane 6), and few progeny appeared (Table 1). Early and late protein synthesis as well as progeny production were similar in *rpoH* mutant cells and *rpoH*⁺ cells grown and infected at 30°C (Fig. 1 and Table 1).

To test whether these effects correlate with altered transcription, we analyzed, as an example, the transcripts of the gene 69-*oriA* region with a probe that does not hybridize to late transcripts (Fig. 2) (12). In this region, a 1,050-nucleotide transcript is initiated from an early promoter and a 600-nucleotide transcript is initiated from a middle promoter (12). Figure 2 shows that the abundance of both transcripts was greatly reduced when *rpoH*⁺ cells were shifted to 42°C at the time of infection. As expected, the abundance of these transcripts was reduced in *rpoH* mutant cells regardless of the time of temperature shift. Together, these results indicate that a successful heat shock response is required for T4 early transcription at high temperature. They are consistent with the idea that sigma 32 out-competes sigma 70 upon temperature shift (6, 7, 16, 19, 25) and that *rpoH* mutant cells

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FIG. 1. T4 proteins synthesized when host cells were shifted to 42°C before or at the time of infection. *rpoH*⁺ and *rpoH* mutant cells (SC122 and K165, respectively; a gift of F. Neidhardt) were grown at 30°C to 10⁸ cells per ml, concentrated to 10⁹ cells per ml, and infected with 10 wild-type T4 particles per cell. At the indicated times, cells were shifted from 30 to 42°C. Control samples remained at 30°C. Infected cells were pulse-labeled with ³H-labeled L-amino acids (specific activity, 5 μCi/ml) for two different times after infection (indicated at top) and chased with excess Casamino acids. Infected cells were then lysed in Laemmli sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer (11). Labeled proteins were separated on 20% "low bis" polyacrylamide gels at pH 7.8 with a 5% stacking gel (pH 6.8) (1). Lanes: 1, *rpoH*⁺ cells shifted to 42°C at the time of infection; 2, *rpoH* mutant cells shifted to 42°C at the time of infection; 3, *rpoH*⁺ cells grown and infected at 30°C; 4, *rpoH* mutant cells grown and infected at 30°C; 5, *rpoH*⁺ cells shifted to 42°C 30 min before infection; 6, *rpoH* mutant cells shifted to 42°C 30 min before infection.

FIG. 2. Northern blot of early T4 RNA probed with the gene 69-oriA region of T4. *rpoH*⁺ and *rpoH* mutant cells were infected with wild-type T4 under the conditions described in the legend to Fig. 1. RNA isolated from infected cells at 8 min after infection was separated and blotted as previously described (22). It was probed with RNA transcribed by SP6 RNA polymerase (Bethesda Research Laboratories, Inc.) from an *EcoRI* DNA fragment cloned into pSP64 (Promega Biotech). Transcription conditions were according to the directions of Promega Biotech. Infection conditions were as follows: lane 1, *rpoH*⁺ cells grown and infected at 30°C; lane 2, *rpoH* mutant cells grown and infected at 30°C; lane 3, *rpoH*⁺ cells infected at 30°C and immediately shifted to 42°C; lane 4, *rpoH* mutant cells infected at 30°C and immediately shifted to 42°C; lane 5, *rpoH*⁺ cells shifted to 42°C 30 min before infection; lane 6, *rpoH* mutant cells shifted to 42°C 30 min before infection. The T4 transcripts of 1,050 and 600 nucleotides are marked with arrows, and the host rRNAs of 2,960 and 1,675 nucleotides are marked with arrowheads.

TABLE 1. T4 progeny production per infected cell					
Host	Infecting phage	Progeny per infected cell after shift at time ^a :			
		-30 min	0 min	10 min	No shift ^b
<i>rpoH</i> ⁺	Wild type	43	2	50	85
	<i>dell2</i>	29	4	40	44
<i>rpoH</i>	Wild type	6	3	7	90
	<i>dell2</i>	0.06	0.12	33	39

^a Shift from 30 to 42°C at time after infection.
^b No shift (30°C).

fail to synthesize additional sigma 70 in response to heat shock.

Effects of heat shock at late times after infection. When infected *rpoH*⁺ cells were shifted to a higher temperature 10 min after infection, at a time when T4 sigma gp55 had been synthesized, T4 late protein synthesis appeared normal (Fig. 3). In contrast, under these conditions only small amounts of late proteins were synthesized in *rpoH* mutant cells (Fig. 3), and the burst size was low (Table 1). The defective late protein synthesis under *rpoH* mutant conditions correlated with low levels of the corresponding transcripts (Fig. 4). These results suggest that at 42°C T4 late gene expression requires a functional *E. coli* heat shock sigma factor in an unknown capacity. A few T4 proteins, most notably those encoded by *uvsX* and *uvsY* as well as gp32, are synthesized in abundance under these conditions, probably because they are translated from stable prereplicative messages (5, 8). The fact that the *uvsY* transcripts, which are known to be stable prereplicative messages (8), were stable under these conditions (Fig. 4) indicates that the low level of late transcripts is not due to excessive, general degradation of mRNA. DNA synthesis in infected *rpoH*⁺ and in *rpoH* mutant cells heat shocked 10 min after infection was normal and indistinguishable (data not shown). Together, these results show that both *rpoH*⁺ and *rpoH* mutant cells are metabolically active under these conditions and that the lack of late proteins in *rpoH* mutant cells is not due to general decay of cellular functions, to degradation of T4 DNA, or to defective replication. One of the most convincing demonstrations of the metabolic potential of the *rpoH* mutant cells under these conditions is the synthesis of late proteins when these cells are infected with the T4 deletion mutant, *del(39-56)12*, which lacks more than 10 kilobases of nonessential T4 DNA (9) (Fig. 3). This result shows that *rpoH* mutant cells heat shocked 10 min after infection are able to support T4 late gene expression and suggests that a phage factor encoded within the deleted region renders sigma 32 essential for late transcription at high temperatures. Kassavetis and Geiduschek have shown that gp55 is the only RNA polymerase sigma factor required for the recognition of the T4 late gene 23 promoter in vitro at 37°C (10). Our findings suggest a complex interaction of several host and phage factors involved in the regulation of T4 late gene expression in vivo. We are now trying to identify the putative factor(s), encoded in the nonessential region, that renders sigma 32 essential at 42°C.

Since our experiments were done under conditions that prevented T4 transcription, they could not detect additional effects of *E. coli* heat shock proteins on subsequent steps of T4 development. For example, *rpoH*⁺ cells that are heat shocked 5 min after infection do synthesize late T4 proteins, but these proteins are incorrectly assembled. The defect can be partially corrected by overproduction from a plasmid of the *E. coli* GroEL protein, a heat shock protein required for normal T4 assembly (24).

Our results provide an explanation for many differences in results obtained with the same *ts* mutants under different conditions of infection. For example, T4 gene 32 mutants, which had been classified as being completely defective in DNA replication (18), undergo at least one round of DNA replication when cells are adapted to 42°C before infection (14).

Our unexpected observation that, at high temperature, development of phage T4 is affected by *E. coli* sigma factors 70 and 32, illustrates the complex interaction of this molecular parasite with its host throughout the viral life cycle.

FIG. 3. T4 proteins pulse-labeled 15 to 20 min after infection when temperature was shifted to 42°C 10 min after infection. Cells were grown at 30°C to 10^8 cells per ml, concentrated to 10^9 cells per ml, and infected with 10 T4 particles per cell. The proteins were separated as described in the legend to Fig. 1. Lanes: 1, *rpoH* mutant cells shifted to 42°C 10 min after infection; 2, control infected *rpoH* mutant cells incubated at 30°C; 3, *rpoH*⁺ cells shifted to 42°C 10 min after infection; 4, infected *rpoH*⁺ cells incubated at 30°C; 5, *rpoH* mutant cells infected with *del(39-56)12* T4 and shifted to 42°C 10 min after infection; 6, *rpoH* mutant cells infected with wild-type T4 and shifted to 42°C 10 min after infection.

FIG. 4. Dot blots of T4 RNA isolated at 20 min after infection. Cells were grown to 3×10^8 cells per ml and infected with 10 wild-type T4 particles per cell. RNA was isolated as described (legend of Fig. 2). Total RNA was blotted into a nylon membrane in dots of 0.25, 0.5, and 1.0 μ g (lanes a, b, and c, respectively) as described (3). Gene-specific probes shown were prepared as described (legend of Fig. 2), transcribing pGL737 (gene 23), pGL7 (gene 7), pGL541 (gene 18) (all obtained from G. Lin, this laboratory), and pMGr800 (*uvs Y*; M. Guidl, this laboratory). Conditions were: lane 1, *rpoH*⁺ cells shifted to 42°C 10 min after infection; lane 2, *rpoH* mutant cells shifted to 42°C 10 min after infection; lane 3, infected *rpoH*⁺ cells incubated at 30°C; lane 4, infected *rpoH* mutant cells incubated at 30°C.

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