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# Involvement of Heat Shock Proteins in Bacteriophage Mu Development

MARTIN PATO,[1,2]* MARY BANERJEE,[1] LUCIE DESMET,[3] AND ARIANE TOUSSAINT[3]

*Department of Molecular and Cellular Biology, National Jewish Center for Immunology and Respiratory Medicine,[1]* and Department of Microbiology and Immunology, University of Colorado Health Science Center,[2] Denver, Colorado 80206, and Laboratoire de Génétique, Université Libre de Bruxelles, Brussels, Belgium[3]*

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**Growth of bacteriophage Mu was severely inhibited at elevated temperature in mutants defective in the heat shock genes *dnaK*, *groEL*, and *groES* and in the *rpoH* (*htpR*) regulatory mutant, but not in mutants defective in the heat shock genes *dnaJ* or *grpE*; growth of a mutant of Mu deficient in functions encoded in the accessory region of the Mu genome was inhibited in the latter two host mutants. Phage production in the *dnaJ* mutant was restored by growth in low-salt medium. The stage in Mu development primarily affected in all except the *groE* mutants was phage late transcription. In contrast, the *groE* mutants did not support growth of Mu at any temperature; neither Mu DNA replication nor transcription was inhibited in these strains, suggesting that *groE* is required for phage morphogenesis as observed with several other coliphages.**

Upon induction of a Mu lysogen, a series of events occurs culminating in production of progeny bacteriophage. These include (i) transcription of the early region of the Mu genome, (ii) replicative transposition of Mu DNA to amplify the number of genome copies, (iii) transcription of the late region of the Mu genome, (iv) morphogenesis of the phage, and (v) lysis of the cell and release of progeny phage. These events require both phage-encoded and host-encoded enzymes (24), including most of the DNA replication machinery of the host cell.

This paper reports the results of a survey of the effects on Mu development of mutations in several host genes which encode heat shock proteins in *Escherichia coli*. The heat shock regulon encompasses a set of at least 17 proteins under the control of a novel sigma factor encoded by the *rpoH* (*htpR*) locus (16). Many of these proteins are known only by their positions on two-dimensional protein gels, but several of them have been identified. Studies on the involvement of host functions in phage λ development have been instrumental in identifying several of the heat shock proteins: products of the *dnaJ* (18), *dnaK* (8), and *grpE* (18) genes have been shown by in vivo studies to be required for λ DNA replication, and in vitro studies have described their role in the initiation step in replication (5, 14); *groES* and *groEL* gene products are required for morphogenesis of λ phage particles (21, 23). The sigma factor encoded by the *rpoH* locus is probably also a heat shock protein, but the regulation of its synthesis is different from that of the other heat shock proteins (22). The effects on host metabolism of mutations in heat shock genes generally are less clear; at extreme high temperatures several of the mutations are lethal and lead to slow cessation of macromolecular syntheses (2, 10, 18, 19, 25). The pattern of inhibition of phage growth in several of the mutants observed in this study is markedly different from that with λ. The stage of Mu development primarily affected by the *dnaJ*, *dnaK*, and *grpE* mutations was late transcription; DNA replication was essentially unaffected, unlike λ development in which the primary effect observed is inhibition of DNA replication. Both transcription and DNA replication were normal in the *groEL* and *groES* hosts, suggesting that the defect in this host is in phage morphogenesis, as is true for λ and other coliphages.

* Corresponding author.

## MATERIALS AND METHODS

**Bacteria.** All strains used were derivatives of *E. coli* K-12. The *dnaK756*, *dnaJ259*, *grpEL280*, and *groES30* mutants and their respective parents were obtained from C. Georgopoulos; the *htpR165* and *groEL44* mutants and their respective parents were from F. Neidhardt; MC4100($\lambda_p$F13-[$P_{groE}$-*lacZ*]) and MC4100 ($\lambda_p$F13-[$P_{rpoDhs}$-*lacZ*]) were from T. Yura.

**Phage.** Mu pAp1 is a Mu *c*ts62 with a substitution of a 1-kilobase (kb) fragment from Tn*3* containing the $Ap^r$ marker for a similar length of DNA from the right end of the Mu G region (13). Mu pAp5 is a Mu *c*ts62 with a similar substitution replacing the region from about 6.2 to 7.2 kb from the Mu left end (1). The phage titers were determined on L agar (L broth with 1% agar) at 37°C.

**Media and growth conditions.** Bacteria were grown in L broth (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl per liter) at 30°C to a density of approximately $10^8$ cells per ml. For induction, samples were transferred to prewarmed flasks at elevated temperatures, generally 43.3°C. For infection, $CaCl_2$ was added to a concentration of 3 mM and the cells were infected with phage at a multiplicity of about 5.

**Measurement of DNA replication and transcription.** At indicated times, 2-ml samples were removed and pulse-labeled for 2 min with [$^3$H]thymidine (50 μC/ml) or [$^3$H]uridine (50 μC/ml) to label DNA or RNA, respectively. The labeling was terminated by dilution into 10 ml of ice-cold stop buffer (10 mM Tris [pH 8.1], 10 mM EDTA, 10 mM NaCN). To determine the amount of incorporation of label, we precipitated 0.1 ml of the diluted samples with cold 10% trichloroacetic acid; the precipitates were collected on nitrocellulose filters and washed, and their radioactivity counted in scintillation fluid. The remainder of the samples was prepared for annealing to Mu DNA immobilized on nitrocel-

lulose filters as described by Waggoner and Pato (26) for DNA and Bovre and Szybalski (4) for RNA.

**Measurement of beta-galactosidase.** Beta-galactosidase synthesized in strains carrying fusions of *lacZ* to the heat shock promoters for *groE* and for *rpoD* (29) was measured as described by Miller (15).

## RESULTS

**Induction of lysogens.** Lysogens of the various heat shock mutants of *E. coli* and the respective parental strains were prepared by infection with Mu pAp1 and selection of ampicillin-resistant, phage-producing cells. Mu pAp1 contains a 1-kb substitution of a fragment of Tn*3* conferring ampicillin resistance near the right end of the Mu genome and behaves normally in all aspects of Mu development that have been examined. The lysogens were grown in L broth at 30°C and induced by shifting the cultures to 43.3°C. Samples were taken after lysis or as late as 120 min after induction, and the titers of the progeny phage were determined. The ratio of the titers of Mu pAp1 on mutant hosts relative to the titers on parental wild-type hosts was determined for each mutant. Phage production was severely inhibited in the *dnaK* ($10^{-3}$ to $10^{-5}$), *rpoH* ($10^{-3}$ to $10^{-4}$), *groEL* ($10^{-4}$), and *groES* ($10^{-4}$) hosts, was essentially normal in the *dnaJ* (1) host, and was slightly reduced in the *grpE* (1 to $10^{-1}$) host.

**Mu DNA replication and transcription in heat shock mutants.** To determine the stage(s) at which phage development is blocked in the mutants which failed to sustain Mu growth, we pulse-labeled lysogens of the mutant and parental strains with [$^3$H]thymidine or [$^3$H]uridine to measure DNA and RNA synthesis at various times after induction at 43.3°C. The heat shock mutations confer temperature sensitivity on host cell growth, and at elevated temperature host macromolecular syntheses in the mutants slowly decrease. In our experiments, host DNA and RNA synthesis were generally reduced about twofold by 40 to 60 min after shifting to 43.3°C (data not shown). The data for Mu-specific macromolecular syntheses shown in Fig. 1 are expressed as the ratio of Mu-specific DNA or RNA synthesis to total DNA or RNA synthesis. The results are summarized below.

***dnaK.*** The data in Fig. 1 (top panels) show that the *dnaK* mutation had no effect on phage DNA replication, in striking contrast to the observations with phage λ. However, phage transcription at late times after induction was severely reduced. Transcription at late times is normally mostly from the late region of the genome (late transcription), with a small amount of continued transcription, about 5%, from the early region (28). The level of authentic late transcription in the *dnaK* mutant is actually about half that shown in Fig. 1, as further analysis of the RNA synthesized at late times showed that about half the transcripts were from the early region (data not shown). We estimate that the decrease in late transcription in the *dnaK* host relative to that in the parental strain was approximately six- to eightfold. Lysis of the cells, mediated by the *lys* gene encoded in the late region, did not occur.

***rpoH* (*htpR*).** The data in Fig. 1 (middle panels) show that Mu DNA replication was reduced in the *rpoH* host relative to that in the parental host and that late transcription was virtually eliminated. No lysis of the cells was observed.

***groEL.*** Figure 1 (lower panels) shows that both Mu DNA replication and late transcription were essentially unaffected in the mutant host relative to the parental host. Lysis of the host occurred, although almost no phage were produced. Identical results were obtained with the *groES* mutant (data not shown).

***dnaJ* and *grpE*.** Little or no effect on Mu growth and macromolecular syntheses was observed with the *dnaJ* and *grpE* mutants (see below and Fig. 2).

***dnaJ*, *grpE*, and Mu phage deficient in the accessory region.** The portion of the Mu genome between the end of the *B* gene (4.3 kb) and the *C* gene (10.0 kb) contains numerous open reading frames (H. Preiss, B. Bräuer, C. Schmidt, and D. Kamp, *in* N. Symonds, A. Toussaint, P. Van de Putte, and M. Howe, ed., *Phage Mu*, in press), and a number of phenotypes have been ascribed to this region. Expression of this region is under the control of the early promoter. Inactivation of the entire region by polar insertions beyond the end of the *B* gene results in viable phage which, however, grow very poorly and plaque with reduced efficiency on most indicator strains; hence, the sobriquet accessory or semiessential. The phage Mu pAp5 contains a deletion from 6.2 to 7.2 kb and an insertion of a polar fragment of Tn*3* containing the gene conferring ampicillin resistance, blocking transcription of the distal portion of the accessory region. This phage produces slightly smaller plaques than Mu pAp1, although phage yields upon induction are similar.

We compared Mu pAp5 with Mu pAp1 in examining the effects of mutations in the heat shock genes on Mu development. In addition to being severely inhibited in growth at elevated temperature in the *dnaK*, *rpoH*, *groEL*, and *groES* mutants, Mu pAp5 was severely inhibited in its growth in the *dnaJ* and *grpE* mutants at 43.3°C: the ratio of Mu pAp5 titer to Mu pAp1 titer in the mutants was about $10^{-3}$ for the *dnaJ* mutant and $10^{-3}$ to $10^{-4}$ for the *grpE* mutant.

Examination of the stage of Mu development affected in the *dnaJ* and *grpE* mutants (Fig. 2) showed that late transcription was primarily affected. We estimate that late transcription of Mu pAp5 in the *dnaJ* host was reduced by more than 10-fold compared with late transcription of Mu pAp1. The slight decrease in the rate of DNA synthesis of Mu pAp5 relative to that of Mu pAp1 (Fig. 2) was also seen with the *dnaJ*$^+$ parent (data not shown), as was a slight decrease in the rate of late transcription. No lysis of the cells was observed.

With the *grpE* mutant, some reduction in late transcription occurred with Mu pAp1 relative to transcription with the parental host (Fig. 2), and a greater reduction occurred with Mu pAp5. Delayed lysis of the cells was seen with Mu pAp5, although very few phage were produced.

**Effect of Mu infection on heat shock proteins.** Since several of the heat shock proteins are required for normal Mu growth, we wished to know whether Mu development itself, without a temperature upshift, could induce the heat shock regulon. To do this, we examined the effects of Mu infection; the effects on the regulon were followed by using fusions between *lacZ* and the heat shock promoters for *groE* or *rpoD* and measuring beta-galactosidase activity at intervals after infection with Mu pAp1. The results in Fig. 3 with the *groE* promoter fusion show that little or no increase in beta-galactosidase activity occurred after infection. The same result was obtained with the *rpoD* promoter fusion (data not shown). In contrast, temperature upshift resulted in a two- to threefold increase in beta-galactosidase activity within 15 min, consistent with a more than 10-fold increase in the rate of synthesis during that time. Hence, Mu development does not induce the heat shock regulon.

**Salt effect on phage production.** The literature contains several examples of suppression of temperature-sensitive mutations by high salt (12). All the lysogens were examined for phage production in normal L broth (85 mM NaCl), high-salt L broth (170 mM NaCl), and low-salt L broth (3

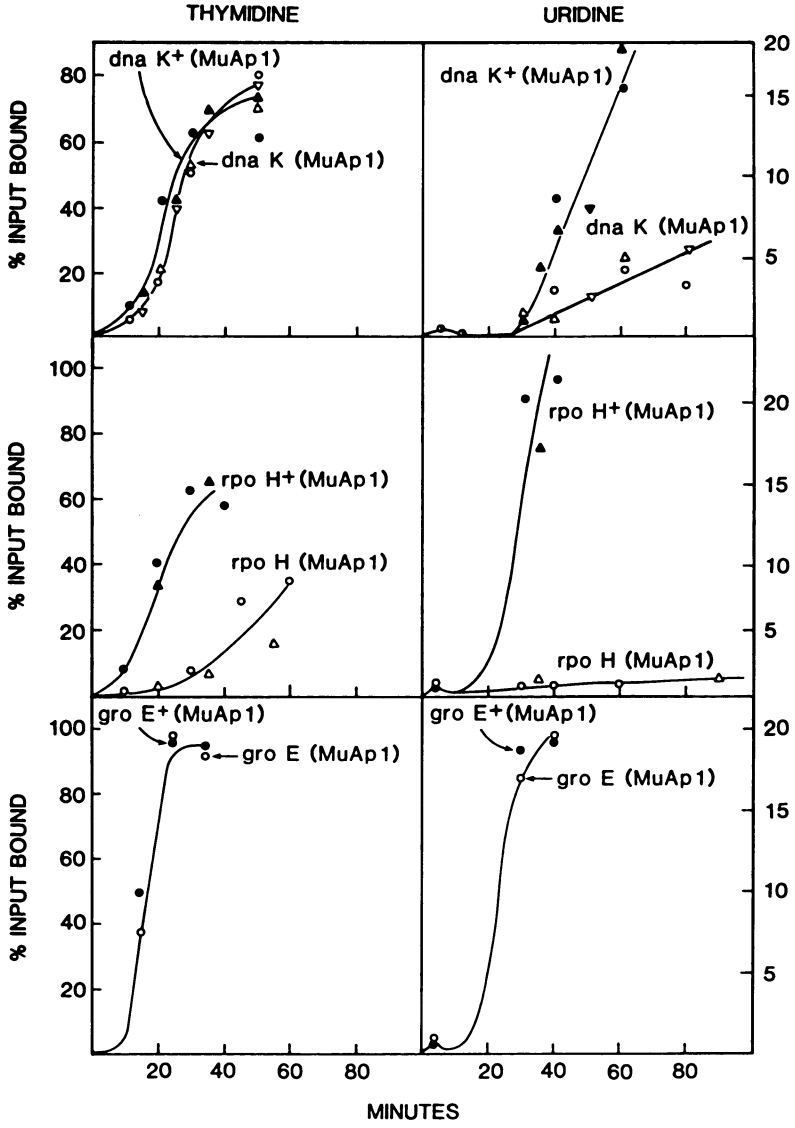


FIG. 1. Replication and transcription of bacteriophage Mu DNA in *dnaK*, *rpoH*, and *groEL* mutants and their respective parental strains. Mu pAp1 lysogens of the mutant (open symbols) and parental (closed symbols) strains were grown at 30°C and induced at 43.3°C. Samples were removed at intervals and pulse-labeled for 2 min with [$^3$H]thymidine or [$^3$H]uridine. DNA and RNA were prepared and annealed to nitrocellulose filters with Mu DNA as described in Materials and Methods. The results are expressed as the percentage of input counts per minute bound to the filters in the annealing assay. Left panels, Replication; right panels, transcription. Different symbols represent different experiments.

mM NaCl). High-salt medium failed to suppress the effects of the mutations in any of the lysogens. However, the inhibition of growth of Mu pAp5 in the *dnaJ* mutant at high temperature was not observed in the low-salt L broth.

## DISCUSSION

Growth of bacteriophage Mu was severely inhibited at elevated temperature in mutants defective in the heat shock genes *dnaK*, *groEL*, and *groES* and in the *rpoH* regulatory mutant, but not in *dnaJ* or *grpE* mutants. Growth of the mutant phage Mu pAp5, deficient in functions encoded in the accessory region, was inhibited in *dnaJ* and *grpE* mutants. Several interesting features of this inhibition pattern require discussion, including the stages of development at which the effects are exerted and the potential role of the accessory functions.

**Developmental stage affected.** Several of the lysogens in which Mu development was inhibited at elevated temperature [*dnaK*(Mu pAp1), *dnaJ*(Mu pAp5), *grpE*(Mu pAp5)] exhibited normal patterns of early transcription and of replication of Mu DNA after induction. However, the levels of late transcription were markedly reduced in each of these lysogens.

The above pattern is thus different from that observed with bacteriophage λ, as in vivo and in vitro studies have clearly shown that several of the heat shock proteins (*dnaJ*, *dnaK*, and probably *grpE*) are required for initiation of λ DNA replication. Dodson et al. (5) and McMacken et al. (14) have defined the role of these proteins to be at a stage in initiation after binding of the λ O and P proteins and host

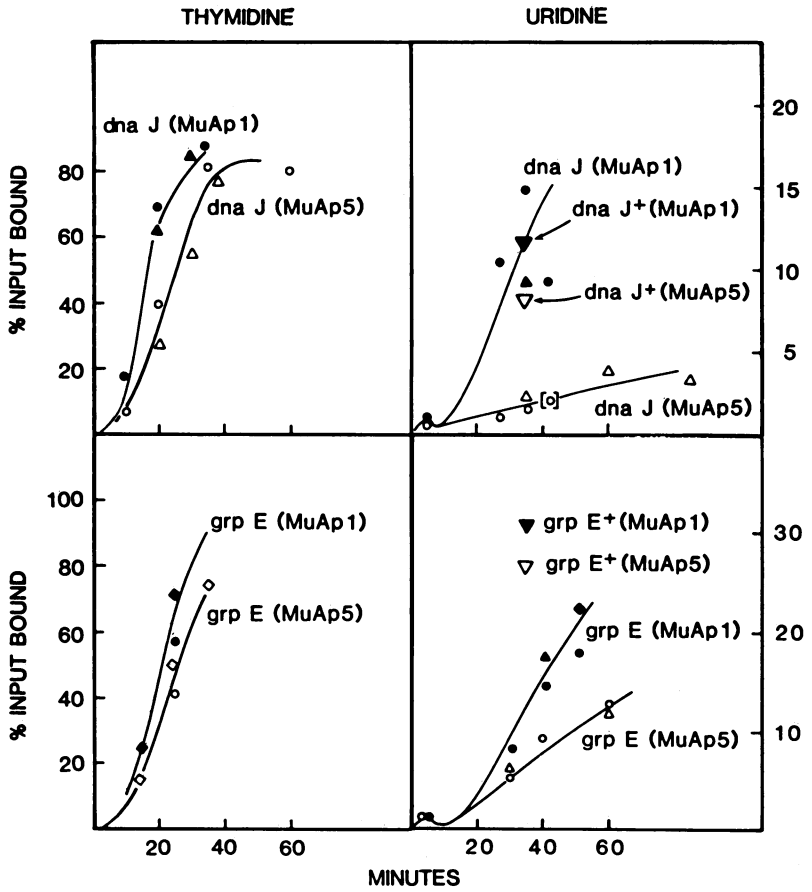


FIG. 2. Replication and transcription of Mu pAp1 and Mu pAp5 in *dnaJ* and *grpE* mutants. Mu pAp1 (closed symbols) and Mu pAp5 (open symbols) lysogens of the mutant strains were induced and labeled as described in the legend to Fig. 1. Different symbols represent different experiments. The large inverted triangles represent experiments with the parental wild-type hosts.

*dnaB* protein to the origin of λ replication. These researchers suggest that the proteins are involved in opening of the DNA helix to form a replication eye, possibly by allowing dissociation of the P protein and activation of the helicase activity of the dnaB protein.

In striking contrast, the *dnaK*, *dnaJ*, and *grpE* proteins are not required for Mu DNA replication, although they are required for Mu development under the appropriate conditions; therefore, their function is not restricted to DNA replication, as already anticipated by the observation that all macromolecular synthesis in *E. coli* is affected at high temperatures in these mutants. The reason that Mu DNA replication does not require these proteins while λ and probably *E. coli* DNA replication does is most likely related to the different mechanisms used to initiate replication in each of these organisms. λ and *E. coli* DNA replication requires a series of reactions resulting in synthesis of a primer RNA at an origin of replication. Initiation of Mu DNA replication, as envisioned by the current model for replicative transposition (20), replaces these events with a series of transposase-catalyzed nicking and ligation reactions which produce an intermediate immediately resembling the structure involved in the propagation stage in DNA replication, i.e., possessing free 3′-hydroxyl ends of DNA which can be used directly by DNA polymerase. It is tempting to speculate that the affected step in Mu development, late transcription, involves a role for these heat shock mutants in opening of the DNA helix as a prerequisite for transcription.

The *groE* mutants were unable to support phage production, even at low temperatures at which the effects of the other heat shock functions were minimal. Mu DNA replication and transcription were normal in these strains, as was lysis of the induced cultures. These observations are consistent with the finding that the *groE* proteins are required for the correct assembly of the virions of several other coliphages (9, 21, 23), and it is likely that the defect in Mu development in these mutants is also in morphogenesis.

**Effects on late transcription.** Control of Mu late transcription is poorly understood at this time. The product of the *C* gene is required, but regulation of its synthesis is not understood. Replication of Mu DNA is also required, as mutations in the *A* or *B* genes or deletions of the termini of the Mu genome—conditions which abolish Mu DNA replication, but not early transcription—all abolish late transcription (27). Similar involvement of phage DNA replication in late transcription has been observed for several phages, such as T4 (7). Although several interesting hypotheses have been proposed to explain this coupling, the mechanism(s) is as yet unknown.

The observation that *dnaJ* mutants can sustain the growth of Mu at elevated temperature, but not of the mutant lacking a functional accessory region, raises the interesting possibility that a protein analogous to the host *dnaJ* protein is encoded in this region. Comparison of the nucleotide se-

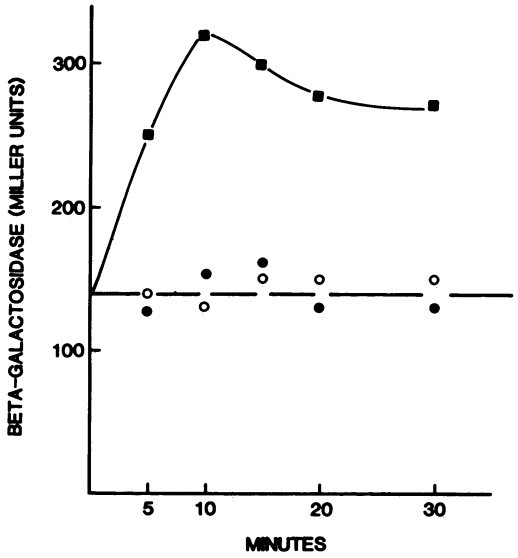


FIG. 3. Beta-galactosidase activity in $P_{groE}$-*lacZ* fusions. *E. coli* MC4100 carrying a $\lambda_P$F13-($P_{groE}$-*lacZ*) prophage was grown at 30°C (●). The cells were infected with Mu pAp1 at a multiplicity of infection of 5 (○) or shifted to 43.3°C (■). Samples were taken at intervals for determination of beta-galactosidase activity and optical density at 550 nm, and the specific enzyme activity was calculated.

quence of *dnaJ* to the portion of the accessory region affected in Mu pAp5 revealed some homology with one of the open reading frames (E13, the 13th open reading frame in the early transcript); the region of homology includes the cysteine-rich consensus sequence described by Berg (3) for potential metal-binding domains in nucleic acid-binding proteins. The presence of such an essential phage-encoded function, which has an analogous function in the host, can be determined only under conditions in which both the host- and phage-encoded functions can be inactivated. To address this question, we are currently examining whether cloned fragments of the accessory region can complement Mu pAp5 growth in a *dnaJ* host.

A similar argument applies to the *grpE* mutation, though the situation with this mutation is somewhat less clear. A slight reduction in phage titer and in late transcription was observed even with the wild-type phage, while severe reduction of phage titer and further reduction of late transcription were observed with Mu pAp5. The reduction of late transcription was insufficient to prevent the cell lysis resulting from *lys* gene expression.

**Stimulation of heat shock protein synthesis.** Little or no increase in expression from the *groE* or *rpoD* promoters was observed in response to Mu infection. Although the assay used is less sensitive than methods which measure rates of synthesis, it is sufficiently sensitive to observe the large stimulations of enzyme content after temperature shift (Fig. 3). The results demonstrate that Mu development does not induce the entire heat shock regulon, but they do not exclude the possibility that the synthesis of a subset of the heat shock proteins is stimulated by Mu infection. In this context, the inhibition of Mu growth in the *rpoH* mutant at high temperature is of interest since this mutation should have a pleiotropic effect on the level of all of the heat shock proteins. A small stimulation of *groE* protein synthesis in response to λ infection has been reported (6, 11), perhaps indicating another difference between λ and Mu.

**Suppression by low salt.** The inhibition of growth of Mu pAp5 at elevated temperature in the *dnaJ* mutant was completely suppressed in low-salt medium. This observation is potentially interesting in that one of the postulated roles of heat shock proteins (17), and of *dnaJ* in particular (5), is in disaggregation of protein complexes, an event that may be facilitated by low ionic strength. Mu late transcription may require such events, normally mediated by the *dnaJ* product or by an analogous phage function encoded in the accessory region.

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