JOURNAL OF BACTERIOLOGY, Mar. 1986, p. 763–770
0021-9193/86/030763-08$02.00/0

Vol. 165, No. 3

Defect in Expression of Heat-Shock Proteins at High Temperature in *xthA* Mutants

KYUNG-HEE PAEK AND GRAHAM C. WALKER*

Biology Department, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received 12 July 1985/Accepted 2 December 1985

***Escherichia coli* mutants lacking exonuclease III (*xthA*) are defective in the induction of heat-shock proteins upon severe heat-shock treatment (upshift from 30 to 50°C) but not mild heat-shock treatment (upshift from 30 to 42°C). We show that this defect is due to the *xthA* mutation by complementation. Furthermore, increasing the gene dosage of *xthA*$^+$ prolongs the synthesis of heat shock proteins seen after a shift to 42°C. Increasing the gene dosage of *htpR*$^+$ partially suppresses the defect of *xthA* mutants in the synthesis of heat-shock proteins at 50°C. When an *xthA* strain was incubated at 42°C before a shift to 50°C, it was then able to carry out the synthesis of heat-shock proteins at 50°C.**

The heat-shock response appears to have been conserved in evolution across the procaryote-eucaryote boundary (25). A specific set of proteins termed the heat-shock proteins is induced when organisms or cells are exposed to high temperature or other environmental stresses. Such induction of heat-shock proteins seems to represent part of a homeostatic response to environmental stress in both eucaryotes and procaryotes.

To date, in *Escherichia coli* K-12, 17 proteins have been identified as heat-shock proteins by two-dimensional gel electrophoresis (20). In general, when cells are shifted from 30 to 42°C, the rates of heat-shock protein synthesis increase between 5- and 20-fold within a few minutes, whereas those of most other proteins are relatively unaffected (29). When cells are shifted to 50°C, protein synthesis consists almost exclusively of heat-shock proteins, since the synthesis of general cellular proteins is greatly decreased. Seven of the 17 heat-shock proteins have been identified as the products of known genes, and they appear to be involved in major macromolecular processes of the cell (20).

Over the past several years, there has been a considerable increase in our understanding of the regulation of the heat-shock phenomenon in *E. coli*. However, the specific physiological significance, if any, of the induction of the heat-shock proteins by several agents is still unknown, as is the nature of the inducing signal(s) for this system. The heat-shock response can also be induced by agents that induce other stress-response systems. For example, certain SOS-inducing agents, such as UV and nalidixic acid, can also induce heat-shock proteins (12), although it is not clear that generation of an inducing signal for heat shock involves any type of DNA damage (28). Oxidizing agents can induce heat-shock genes in *Salmonella typhimurium*, and it has been suggested that conditions that induce the heat-shock response may do so by imposing an oxidation stress on the cell (14).

The *xthA* gene product of *E. coli* encodes a monomeric 28,000-dalton protein which has four known enzymatic activities: it is an AP endonuclease, a 3′-5′ exonuclease specific for duplex DNA, a DNA 3′-phosphatase, and an RNase H (22). Even though these activities could function in DNA metabolism or repair, mutations in *xthA* do not have striking general effects on DNA replication or repair. The only obvious phenotype of *xthA* mutants is exceptional sensitivity to hydrogen peroxide (7). Hydrogen peroxide is known to produce oxidative DNA damage by producing transient radical species (8).

The research described in this paper began as an effort to answer the question of whether the expression of the *xthA* gene is controlled by the heat-shock regulatory circuitry. Although it does not appear to be, our studies led us to discover some new and somewhat unexpected phenomena which may offer some new clues to understanding the heat-shock phenomena in *E. coli*.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and the plasmids used in this study are listed in Table 1. Derivatives containing the *dnaK756* allele were constructed by P1 transduction, using a linked *thr*::Tn*10* for selection. P1 transductions were performed basically as described by Miller (18). The *htpR* gene has been cloned previously onto multicopy plasmids (19, 27). We independently cloned the *htpR* gene as follows. In the case of pKP11, we performed a partial *Sau*3A1 restriction digestion of whole chromosomal DNA, isolated 5- to 7-kilobase fragments from a low-melting-point agarose gel, and ligated this DNA into the *Bam*HI site of pBR322. The ligated DNA was used to transform K165. We selected for Ap^r colonies and screened temperature-resistant ones at 42°C. Plasmid DNA from temperature-resistant colonies was subjected to restriction endonuclease digestion analysis. One clone whose pattern suggested that we had cloned the previously isolated *htpR* gene was chosen for further study and labeled pKP11. To construct pKP12 and pKP13, the 4.7-kilobase *Hin*dIII segment of pKP11 containing *htpR*$^+$ was subcloned into pACYC184, a medium-copy-number plasmid (2), and pSE101, a low-copy-number plasmid (9). pSE101 has the same replication origin as pSC101 (4). Plasmid pSGR3, which overproduces *xthA*$^+$, was kindly provided by Bernard Weiss at Johns Hopkins University.

Media and reagents. LB medium has been previously described (18). Unless otherwise stated, cells were grown in M9 glucose medium. When required, antibiotics were added to the following concentrations: ampicillin (sodium salt) 50 μg/ml; tetracycline, 20 μg/ml; kanamycin sulfate, 25 μg/ml, chloramphenicol, 25 μg/ml. L-[^{35}S]methionine (>600 mCi/mmol) was purchased from New England Nuclear Corp. Molecular weight standards for sodium dodecyl sulfate

* Corresponding author.

TABLE 1. *E. coli* strains and plasmids

Strain or plasmid	Description	Reference or source
Strains		
AB1157	F⁻ *thr-1 leu-6 proA2 his-4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 supE37* λ⁻	A. J. Clark
BW9109	As AB1157, but Δ(*xthA-pncA*)	(17)
BW2001	As AB1157, but *xthA11*	(17)
BW2030	As AB1157, but *xthA11*	(17)
BW9091	As AB1157, but *xthA1*	(17)
BW9093	As AB1157, but *xthA3*	(17)
RB901	F⁻ *thr-1 leu-6 lacY? rpsL31 mtlA* Δ(*recA srl*)*21 lexA51*(Def) *lexA3* (Ind⁻) *sulA11*	R. Brent
GW4731	As BW9109, but *dnaK756*, *thr*::Tn*10*	This paper
CG828	*sup*° *galE dnaK756 thr::Tn10 lacY1 tonA21*	C. Georgopoulos
K165	F⁻ *lac*(Am) *trp*(Am) *pho*(Am) *mal*(Am) *rpsL sup4*(Ts) *htpR*(Am)	(5)
Plasmids		
pSGR3	Ap^r *xthA*$^+$	(23)
pKP11	Ap^r *htpR*$^+$, pBR322 replication origin	This paper
pKP12	Cm^r *htpR*$^+$, pACYC184 replication origin	This paper
pKP13	Km^r *htpR*$^+$, pSC101 replication origin	This paper

(SDS)-polyacrylamide gel electrophoresis were purchased from Bio-Rad Laboratories.

In vivo labeling of proteins and gel electrophoresis. For [^{35}S]methionine labeling, early log cultures grown in M9 glucose medium at 30°C were labeled with 5 μCi of [^{35}S]methionine per ml, final concentration (for one-dimensional gels), or 30 μCi of [^{35}S]methionine per ml, final concentration (for two-dimensional gels), after various treatments (12). The cells were allowed to incorporate radiolabel for 5 min unless otherwise stated and then were rapidly chilled on ice and collected by centrifugation in a microcentrifuge. The pellets were prepared for and the proteins were separated on 10% SDS–polyacrylamide gels as originally described by Laemmli (13). Two-dimensional gel electrophoresis was performed essentially as described by O'Farrell (21). Extracts were prepared as for one-dimensional gels and were then diluted 1:2 with sample dilution buffer. First-dimension isoelectric focusing gels were subjected to 6,400 V for 1 h. The resolving portion of the second dimension was 10% SDS–polyacrylamide gel. Autoradiography was done by exposing the dried polyacrylamide gels to Kodak XAR-5 X-ray film at −70°C.

Other methods. Methods for plasmid DNA isolation, digestion with restriction enzymes, and analysis by agarose gel electrophoresis of DNA fragments were carried out as described previously (16). Plasmid transformations were carried out by using a modification of the $CaCl_2$ method of Mandel and Higa (15). Labeling of plasmid proteins by the maxicell procedure was performed essentially as described by Sancar et al. (24), with the modification previously reported (9).

RESULTS

Comparison of the synthesis of heat-shock proteins between the wild type and *xthA* mutants. When *xthA* mutants were shifted from 30 to 42°C, the basic heat-shock protein synthesis patterns were similar to those of the wild type. However, when these strains were shifted from 30 to 50°C (conditions under which heat-shock genes are expressed almost exclusively in an *xthA*$^+$ host), the synthesis of heat-shock proteins was greatly decreased (Fig. 1). *E. coli* cannot grow at 50°C, but is killed if maintained at this temperature for a period of time. Thus strains carrying *xthA* mutations were defective in the synthesis of heat-shock proteins (including GroEL [molecular weight, 62,000], DnaK [69,000], F84.1 [84,000], and Lon [94,000]) after a severe heat shock but not after a milder heat shock. We checked several *xthA* mutants, including a deletion mutant (strain BW9109), and several other point mutants (strains BW2001, BW2030, BW9091, and BW9093). We mainly used the deletion mutant for further study.

To examine the kinetics of this phenomenon in greater detail, we labeled *xthA*$^+$ and *xthA* strains for 1-min periods at various times after shifting to 50°C (Fig. 2). The very short labeling period makes it likely that the decrease in the amount of label incorporated into heat-shock proteins reflects an actual decrease in the rate of synthesis of these proteins rather than degradation. The decrease in general protein synthesis appears to occur at approximately the same rate in both strains, but the strongly increased synthesis of heat shock proteins is not observed in the *xthA* mutant.

The [^{35}S]methionine-labeled proteins synthesized by *xthA*$^+$ and *xthA* strains after a 50°C heat shock were separated by two-dimensional gel electrophoresis. The positions

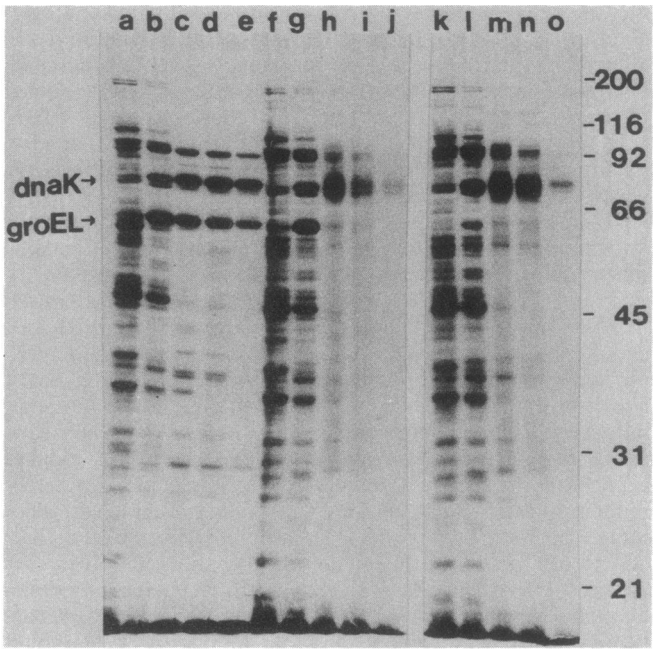

FIG. 1. Comparison of proteins synthesized by *xthA*$^+$ (AB1157) and *xthA* (BW9109 and BW2001) after temperature shifts (30 to 42°C and 30 to 50°C). Cultures were labeled for 5 min. Equal counts were loaded in all lanes. Molecular weights (10^{-3}) of standard proteins are indicated on the right. Arrows indicate the positions of the *groEL* and *dnaK* gene products. The time of labeling after temperature shift is indicated in parentheses. Lanes: (a) AB1157 control, 30°C; (b) AB1157, 42°C (5 min); (c) AB1157, 50°C (5 min); (d) AB1157, 50°C (10 min); (e) AB1157, 50°C (30 min); (f) BW9109 control, 30°C; (g) BW9109, 42°C (5 min); (h) BW9109, 50°C (5 min); (i) BW9109, 50°C (10 min); (j) BW9109, 50°C (30 min); (k) BW2001 control, 30°C; (l) BW2001, 42°C (5 min); (m) BW2001, 50°C (5 min); (n) BW2001, 50°C (10 min); (o) BW2001, 50°C (30 min).

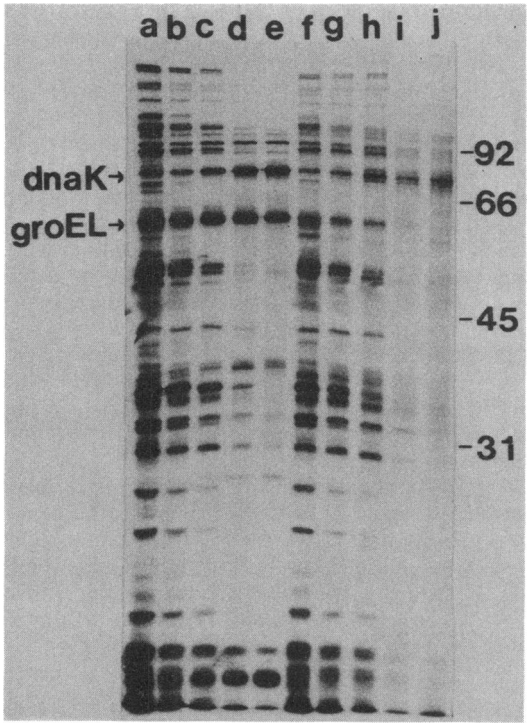

FIG. 2. Comparison of proteins synthesized by AB1157 and BW9109 after a temperature shift from 30 to 50°C. Cultures were labeled for 1 min at various times after shifting to 50°C. Equal counts were loaded in all lanes, but less total protein was loaded per lane than in Fig. 1. Molecular weights (10^{-3}) of standard proteins are indicated on the right. Arrows indicate the positions of the *groEL* and *dnaK* gene products. The time of labeling after temperature shift is indicated in parentheses. Lanes: (a) AB1157, 50°C (1 min); (b) AB1157, 50°C (2 min); (c) AB1157, 50°C (3 min); (d) AB1157, 50°C (5 min); (e) AB1157, 50°C (10 min); (f) BW9109, 50°C (1 min); (g) BW9109, 50°C (2 min); (h) BW9109, 50°C (3 min); (i) BW9109, 50°C (5 min); (j) BW9109, 50°C (10 min).

of GroEL, DnaK, and other heat-shock proteins after a similar separation have been described previously (19). The results (Fig. 3) clearly show that the synthesis of GroEL and several other heat-shock proteins is greatly diminished in *xthA* mutants. Some of the heat-shock proteins are apparently completely missing. Interestingly, in the DnaK protein region, there is much less protein at the normal isoelectric point for DnaK, but at a much more acidic isoelectric point there is a spot which has molecular weight similar to that of DnaK. This may account for some of the blurring of the DnaK region observed on a one-dimensional SDS-polyacrylamide gel. We do not yet know whether this is a modified version of the DnaK protein. In addition it appears that, in *xthA* mutants at 50°C, at least some of the newly synthesized proteins are heterogeneous with respect to size and isoelectric point and that this may also contribute to

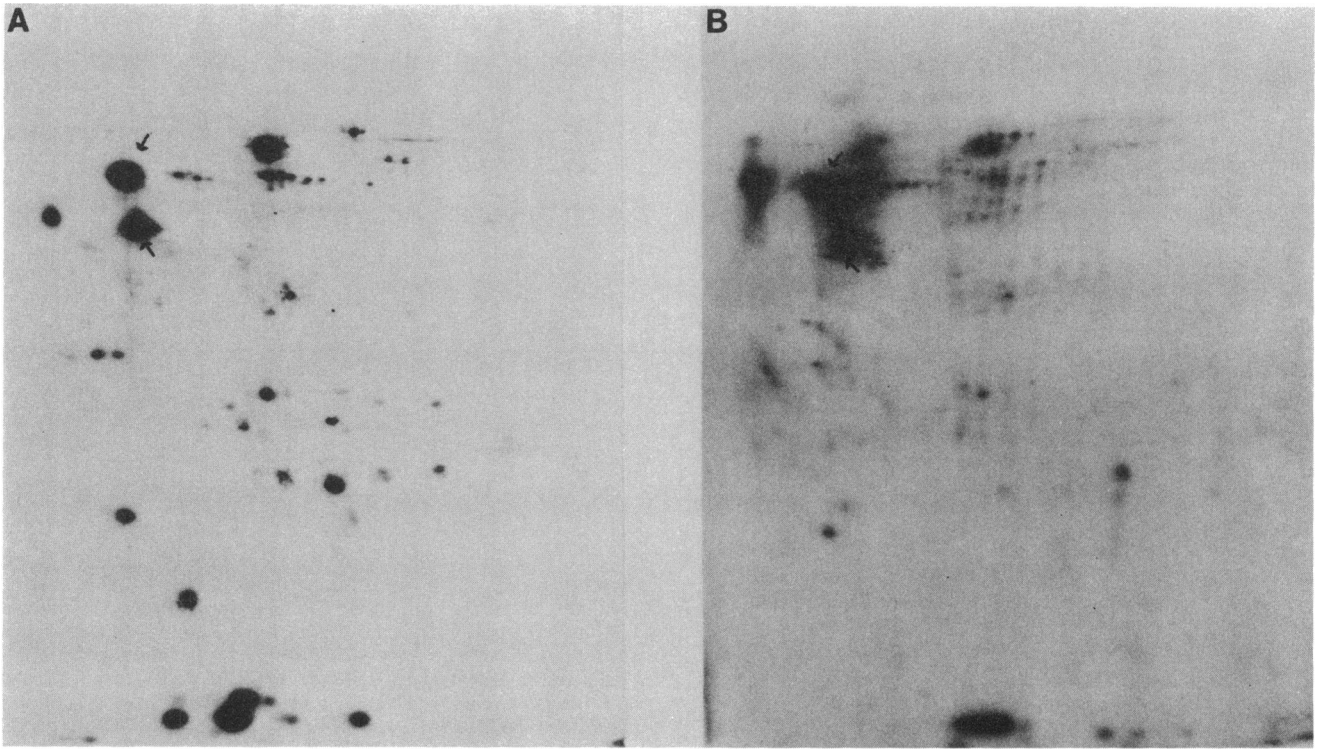

FIG. 3. Two-dimensional gel electrophoresis of [^{35}S]methionine-labeled extracts of AB1157 (A) and BW9109 (B) after a temperature shift from 30 to 50°C. Cultures were labeled for 5 min. Equal amounts of acid-precipitable counts were applied to each gel. Isoelectric focusing was in the horizontal dimension (acidic side is on the left), and SDS-polyacrylamide gel electrophoresis was in the vertical dimension (largest proteins are at the top). Arrows indicate the positions of the *groEL* gene product (M_r, 62,000) and *dnaK* gene product (M_r, 69,000).

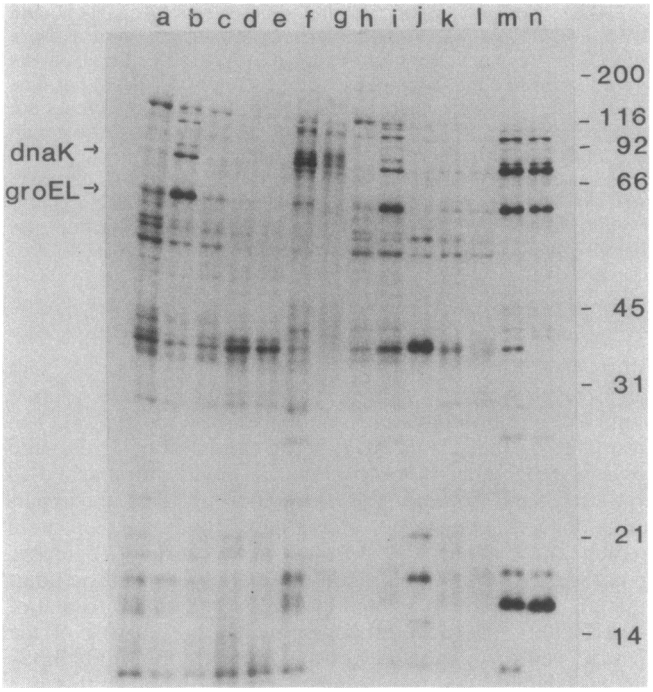

FIG. 4. Proteins from BW9109 (without pSGR3) and BW9109 (with pSGR3) after temperature shifts. Cultures were labeled for 5 min. Equal counts were loaded in all lanes. Molecular weights (10^{-3}) of standard proteins are indicated on the left. *groEL* and *dnaK* gene products are indicated on the right. The time of labeling after temperature shift is indicated in parentheses. Lanes: (a) BW9109 control, 30°C; (b) BW9109, 42°C (5 min); (c) BW9109, 42°C (1 h); (d) BW9109, 42°C (2 h); (e) BW9109, 42°C (3 h); (f) BW9109, 50°C (5 min); (g) BW9109, 50°C (10 min); (h) BW9109(pSGR3) control, 30°C; (i) BW9109(pSGR3), 42°C (5 min); (j) BW9109(pSGR3), 42°C (1 h); (k) BW9109(pSGR3), 42°C (2 h); (l) BW9109(pSGR3), 42°C (3 h); (m) BW9109(pSGR3), 50°C (5 min); (n) BW9109(pSGR3), 50°C (10 min).

some of the blurring seen on one-dimensional gels. It is not yet clear whether the heterogeneity arises from the synthesis of incomplete proteins or from some type of posttranslation modification or processing.

Since ethanol (19), nalidixic acid and UV (12), and hydrogen peroxide (3) also mimic heat shock in inducing some of the heat-shock proteins in *E. coli* and *S. typhimurium*, we examined the response of *xthA* mutants to these agents. Little or no difference was observed in the protein induction pattern between the *xthA* mutants and the wild type (data not shown). Therefore the *xthA* gene product does not seem to be necessary for the induction of heat-shock proteins by these agents. This indicates that *xthA* mutations only affect heat-shock protein synthesis after a severe temperature shift and suggests that some aspect of severe heat shock may differ from milder heat shock and exposure to other stress-inducing agents.

Complementation with a plasmid carrying *xthA*$^+$. To confirm that the defect in heat-shock protein synthesis at high temperature in *xthA* mutants is due to the *xthA* mutations in these strains, we introduced pSGR3, a plasmid that overproduces the *xthA* gene product (23) into a Δ(*xthA-pncA*) strain. We then compared the heat-shock protein synthesis pattern at 50°C in this *xthA* mutant with and without the *xthA*$^+$ plasmid (Fig. 4). By comparing lanes f and g with lanes m and n, it can be seen that the heat-shock proteins are induced by 50°C heat shock only in the presence of this plasmid. This experiment also led to the identification of an unexpected phenomenon. Lanes e and l show the pattern of heat-shock protein synthesis in these strains after a 3-h 42°C heat shock. Normally, with such a long 42°C heat shock, heat-shock protein synthesis diminishes to low level (26); *xthA*$^+$ and *xthA* mutants behave identically in this respect (data not shown). However, in the presence of the multicopy *xthA*$^+$ plasmid, the level of heat-shock protein synthesis continued at an elevated rate.

Plasmids which carry the *htpR*$^+$ gene partially suppress the deficiency of *xthA* mutants in the synthesis of heat-shock proteins. In *E. coli*, the heat shock phenomenon is dependent on the regulatory locus *htpR* (19). K165, a strain carrying an amber mutation in *htpR* (5) shows greatly decreased heat-shock protein synthesis at high temperature (25). Recently evidence has been reported suggesting that the *htpR* protein acts as a sigma factor that promotes transcription initiation at heat-shock promoters (11).

When *htpR*$^+$-containing plasmids of differing copy number were introduced into a Δ(*xthA-pncA*) strain, the heat-shock protein synthesis at 50°C was restored to some extent (Fig. 5). The levels of GroEL provide a preliminary suggestion that this effect might be *htpR*$^+$ gene dosage dependent.

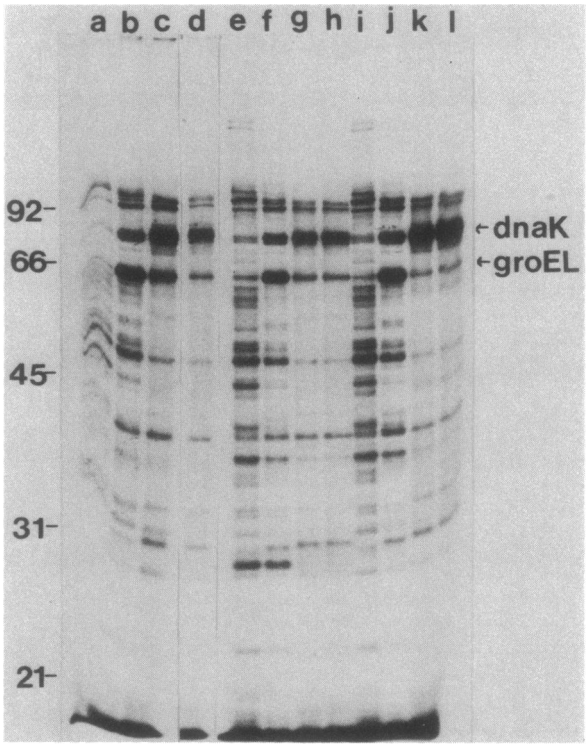

FIG. 5. Comparison of proteins from BW9109 derivatives with *htpR*$^+$ plasmids after temperature shifts. Cultures were labeled 5 minutes. Molecular weights (10^{-3}) of standard proteins are indicated on the left. Arrows indicate the positions of the *groEL* and *dnaK* gene products. The time of labeling after temperature shift is indicated in parentheses. Lanes (a) BW9109(pKP11) control, 30°C; (b) BW9109(pKP11), 42°C (5 min); (c) BW9109(pKP11), 50°C (5 min); (d) BW9109(pKP11), 50°C (10 min); (e) BW9109(pKP12) control, 30°C; (f) BW9109(pKP12), 42°C (5 min); (g) BW9109(pKP12), 50°C (5 min); (h) BW9109(pKP12), 50°C (10 min); (i) BW9109(pKP13) control, 30°C; (j) BW9109(pKP13), 42°C (5 min); (k) BW9109(pKP13), 50°C (5 min); (l) BW9109(pKP13), 50°C (10 min).

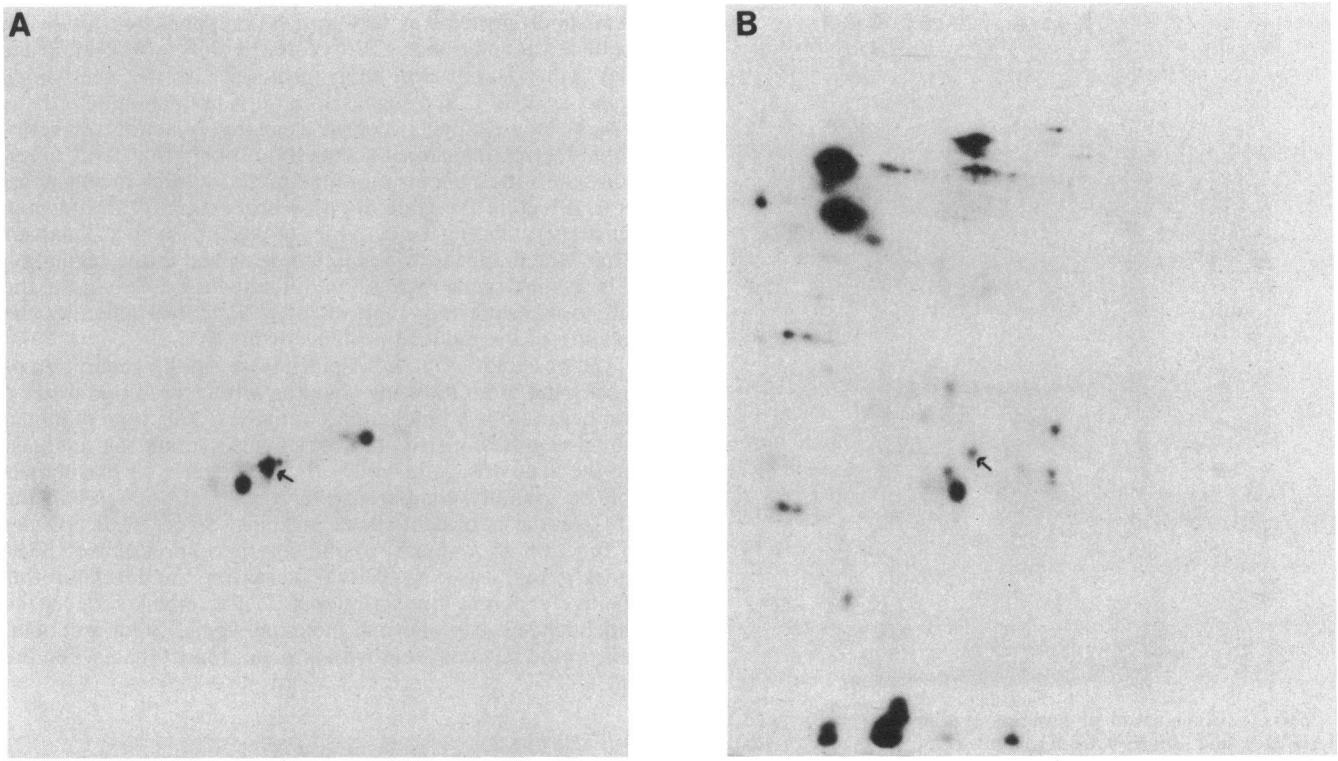

FIG. 6. Two-dimensional gel electrophoresis of [^{35}S]methionine-labeled extracts. The horizontal dimension was isoelectric focusing (acidic side is on the left), and the vertical dimension was SDS-polyacrylamide gel electrophoresis (largest proteins are at the top). Arrow indicates the position of the *xthA* gene product (M_r, 28,000). (A) maxicell extracts of a pSGR3-containing RB901 strain; (B) maxicell extracts plus whole cell extracts of 50°C heat-induced AB1157.

The *xthA* gene product itself is not a heat-shock protein. We next investigated whether the *xthA* gene product is itself a heat-shock protein. To identify the *xthA* gene product, we introduced the *xthA*$^+$ plasmid pSGR3 into strain RB901 [*recA lexA51* (Def)] and prepared maxicells (24). The ^{35}S-labeled plasmid-coded proteins were separated on a two-dimensional gel. We established the identity of the XthA spot by comparing it with vector-coded proteins and published position of the *xthA*$^+$ gene product on two-dimensional gels (1). To investigate whether this *xthA*$^+$ gene product was a heat-shock protein, we mixed the ^{35}S-labeled maxicell extract of a pSGR3-containing RB901 strain and an ^{35}S-labeled whole cell extract of a 50°C heat-induced wild-type strain. The proteins in this mixture were separated on two-dimensional gels, autoradiographed, and compared with the protein synthesis pattern of a 50°C heat-induced sample only. The spot of the *xthA*$^+$ gene product did not coincide with any of the heat-shock proteins (compare Fig. 3A with Fig. 6). Thus the *xthA* gene product is not itself a heat-shock protein.

Effect of a *dnaK* mutation on heat-shock protein synthesis in an *xthA* mutant. When *E. coli* is exposed to a 42°C heat-shock, the heat-shock protein synthesis is transitory. That is, the synthesis of heat-shock protein reaches its maximum after about 7 min and diminishes to lower level after 15 min of incubation at high temperature (20, 29). Bacteria carrying the *dnaK756* mutation fail to turn off the heat-shock response at high temperature. Instead, they continue to synthesize the heat-shock proteins in large amount and underproduce other proteins (26).

Since after a 50°C heat shock of an *xthA* mutant we always could still see a residual amount of labeled DnaK by SDS-polyacrylamide gel electrophoresis, we decided to look at the effect of a *dnaK* mutation in an *xthA* background. To construct a *dnaK xthA* double mutant we transduced the *dnaK756* mutation into the Δ(*xthA-pncA*) strain BW9109 by using a *thr*::Tn*10* mutation linked to *dnaK756*. The presence of the *dnaK756* mutation in the double mutant was judged by the inability of the strain to propagate bacteriophage lambda and form colonies at 43.5°C. The results obtained with the *dnaK xthA* strain are shown in Fig. 7 and 8. At 50°C, the presence of the *dnaK756* mutation did not alter the pattern of protein synthesis compared to a simple *xthA* mutant. In contrast, at 42°C, the presence of the *dnaK756* mutation led to a prolonged overproduction of the heat-shock proteins. These results suggest that the deficiency of *xthA* mutants in the synthesis of heat shock proteins at 50°C is not the result of a premature shutdown of heat-shock mRNA synthesis mediated by DnaK.

Adaptation at 42°C. Since *xthA* mutants exhibited a normal heat-shock response after a shift to 42°C but not after a shift to 50°C, we tested the effect of a preincubation period at 42°C on protein synthesis after a shift to 50°C. In an *xthA*$^+$ strain, the heat-shock proteins were synthesized in abundant amounts at a fast rate after a 5-min adaptation period at 42°C. In contrast, in an *xthA* mutant, a short adaptation at 42°C did not restore the synthesis of heat-shock proteins at 50°C. However, after a longer adaptation at 42°C (about 30 min) the heat-shock protein synthesis at 50°C in an *xthA* mutant was restored fully and increased even more up to a 2-h adaptation period (Fig. 9). Thus heat-shock proteins can be produced at 50°C in *xthA* mutants if they are adapted long enough at 42°C. This observation suggests that the defect of heat-shock protein synthesis at 50°C in *xthA* mutants is due

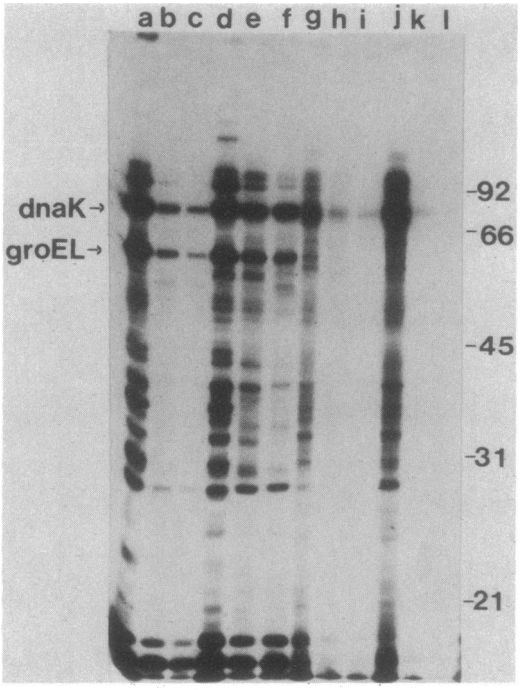

FIG. 7. Comparison of proteins synthesized by the wild type (AB1157) and *dnaK* (CG828), *xthA* (BW9109), and *dnaK xthA* (GW4731) mutants after a temperature shift from 30 to 50°C. Cultures were labeled for 5 min. Molecular weights (10^{-3}) of standard proteins are indicated on the right. Arrows indicate the positions of the *groEL* and *dnaK* gene products. The time of labeling after temperature shift is indicated in parentheses. Lanes: (a) AB1157, 50°C (5 min); (b) AB1157, 50°C (10 min); (c) AB1157, 50°C (30 min); (d) CG828, 50°C (5 min); (e) CG828, 50°C (10 min); (f) CG828, 50°C (30 min); (g) BW9109; 50°C (5 min); (h) BW9109, 50°C (10 min); (i) BW9109, 50°C (30 min); (j) GW4731, 50°C (5 min); (k) GW4731, 50°C (10 min); (l) GW4731, 50°C (30 min).

to an inability to respond quickly to a sudden, severe change of temperature rather than to an absolute defect in the synthesis of heat-shock proteins at 50°C.

DISCUSSION

The results presented in this paper indicate that *xthA* function is required for the synthesis of heat-shock proteins in cells which have been shifted from 30 to 50°C. There is not a general requirement for *xthA* for the synthesis of heat-shock proteins after all inducing treatments, since *xthA* mutants apparently induce the heat-shock proteins normally after a 30 to 42°C shift as well as after treatments with UV, ethanol, and hydrogen peroxide. Rather, the need for *xthA* function seems to be confined to a situation in which cells have undergone a particularly severe heat shock. Our results underscore the conclusion that, even though a number of different stress-inducing agents induce the synthesis of at least some heat-shock genes, there are distinct differences in cellular responses to different types of stress. In the course of these experiments, we also noted that, in *xthA* mutants shifted from 30 to 50°C, at least some of the newly synthesized proteins are heterogeneous with respect to size and isoelectric point (Fig. 3). It is not clear whether this heterogeneity arises from the synthesis of incomplete proteins or from some type of posttranslation modification or processing.

The fact that the defect of *xthA* mutants in the synthesis of heat-shock proteins at 50°C can be compensated for by an extended adaptation at 42°C before the shift to 50°C indicates that XthA is not absolutely required for the heat-shock response at 50°C and suggests that it is not acting directly as a regulatory element, such as a positively acting transcriptional factor. Instead, it seems more likely that *xthA* mutations exert their effects more indirectly by such mechanisms as (i) affecting the generation or processing of an inducing signal, (ii) affecting the activities of proteins such as DnaK or HtpR which appear to be directly involved in the regulation of heat-shock gene expression, (iii) affecting the ability of the cell to translate any type of mRNA, or (iv) affecting the stability of the induced proteins or mRNA.

The possibility that XthA participates in the generation or processing of an inducing signal is attractive, since it has a number of known biochemical activities. This type of model would also be consistent with our observation that increasing the gene dosage of $htpR^+$ by providing it on plasmids is able to partially compensate for the defect of *xthA* in the 50°C synthesis of heat-shock proteins. Since HtpR appears to function as a sigma subunit for the expression of heat-shock genes, it is possible that increasing the dosage of this positively acting transcriptional factor might reduce the dependence on the normal inducing signal. Such a conclusion would be consistent with a recent report that, when the

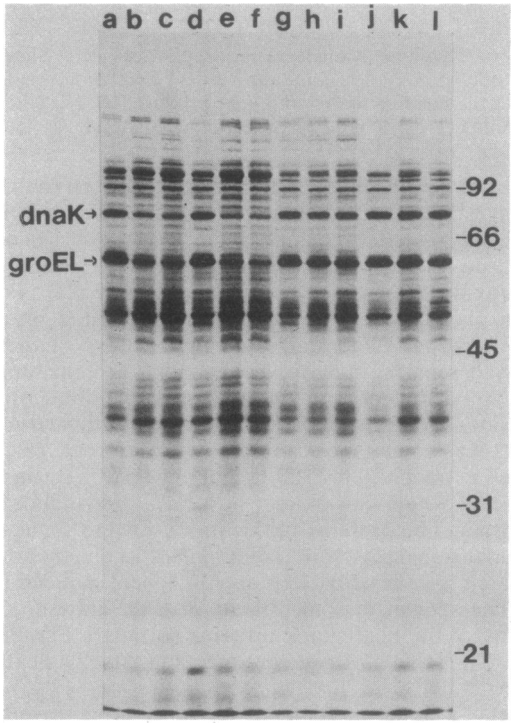

FIG. 8. Comparison of proteins synthesized by the wild type (AB1157) and *xthA* (BW9109), *dnaK* (CG828), and *dnaK xthA* (GW4731) mutants after a temperature shift from 30 to 42°C. Cultures were labeled for 5 min. Molecular weights (10^{-3}) of standard proteins are indicated on the right. Arrows indicate the positions of the *groEL* and *dnaK* gene products. The time of labeling after temperature shift is indicated in parentheses. Lanes: (a) AB1157, 42°C (5 min); (b) AB1157, 42°C (30 min); (c) AB1157, 42°C (1 h); (d) BW9109, 42°C (5 min); (e) BW9109, 42°C (30 min); (f) BW9109, 42°C (1 h); (g) CG828, 42°C (5 min); (h) CG828, 42°C (30 min); (i) CG828, 42°C (1 h); (j) GW4731, 42°C (5 min); (k) GW4731, 42°C (30 min); (l) GW4731, 42°C (1 h).

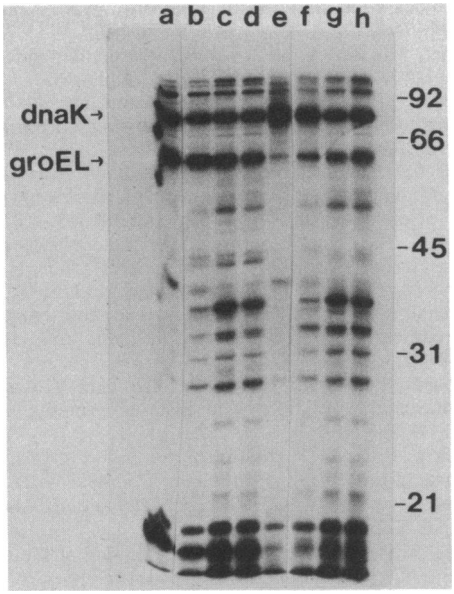

FIG. 9. Comparison of proteins synthesized by *xthA*$^+$ (AB1157) and *xthA* (BW9109) after temperature shifts. Cultures were grown at 30°C until log phase, and then samples were shifted to 42°C. After various times, the cultures were shifted to 50°C and labeled with [^{35}S]methionine for 5 min. Molecular weights (10^{-3}) of standard proteins are indicated on the right. Arrows indicate the positions of the *groEL* and *dnaK* gene products. The 42°C incubation period before shift to 50°C is indicated in parentheses. Lanes: (a) AB1157 (5 min); (b) AB1157 (30 min); (c) AB1157 (1 h); (d) AB1157 (2 h); (e) BW9109 (5 min); (f) BW9109 (30 min); (g) BW9109 (1 h); (h) BW9109 (2 h).

htpR$^+$ gene product is overproduced from a temperature-inducible plasmid after heat shock, the magnitude of the heat-shock response is greater than that in wild-type cells (11). It is also consistent with a variety of studies we have carried out examining the influence of *htpR*$^+$ gene dosage on the expression of the *dnaK* gene in maxicells (unpublished data).

The fact that the known biochemical activities of XthA are related to the processing of nucleic acids raises the possibility that nucleic acids may be involved in the inducing signal. The possibilities for an inducing signal include not only the production of some specific class of structure (in the manner in which single-stranded DNA seems to play a crucial role in SOS induction) but also the production of what might better be termed an induced state, such as a cell with an alteration in the folding or supercoiling of its chromosome.

Speculations concerning the possible involvement of one or more specific biochemical activities of the XthA protein in the phenomena described in this paper are complicated by the fact that *E. coli* has several proteins having certain of the specific activities of XthA. *E. coli* encodes several AP endonucleases, exonucleases, and phosphatases and has another RNase H activity. This redundancy suggests a possible explanation for why *xthA* is not required for the induction of the heat-shock response at 42°C and for how a period of 42°C incubation might compensate for the deficiency in synthesizing heat-shock proteins at 50°C. If a protein having an activity equivalent to a crucial XthA activity were relatively thermostable at 42°C but not at 50°C, then an extended incubation at 42°C might allow sufficient inducing signal(s) to be generated to allow heat-shock synthesis to proceed after a subsequent shift to 50°C.

Although we have discussed the possibility that XthA might affect the production of an inducing signal, the other formal alternatives listed above for how an *xthA* mutation might affect the synthesis of heat-shock proteins at 50°C cannot be eliminated. In this regard, it is intriguing that we have noted two possible relationships between *xthA* and *dnaK*. First, our results provide a preliminary suggestion that *xthA* mutations may affect the modification of *dnaK*, and we are investigating this possibility more carefully. Second, we have noted that increasing the gene dosage of *xthA*$^+$ leads to a phenotype (extended synthesis of heat-shock proteins at 42°C) which is shared by *dnaK* mutants. These observations are not inconsistent with the possibility that *xthA* mutations affect the activity of the DnaK protein, which appears itself to be directly involved in the regulation of heat-shock expression.

The observation that increasing the gene dosage of *htpR*$^+$ can partially suppress the defect of *xthA* mutants at 50°C suggests that an *xthA* mutation does not result in a generalized defect in translation at 50°C. However, the possibility remains that an *xthA* mutation does result in a generalized defect in translation, but that this defect can be overcome by sufficient expression of one or some of the heat-shock proteins. Furthermore, this same observation does not exclude the possibility that *xthA* mutations affect the stability of proteins or mRNA, since *htpR* mutations have been shown to have complex effects on protease activities in *E. coli* (10, 11).

The fact that *xthA* mutants are deficient both in hydrogen peroxide-induced damage repair and in induction of heat-shock response at 50°C is intriguing and has led us to consider whether the two phenotypes would be related. Recently, some of hydrogen peroxide-induced proteins have been shown to be a subset of heat-shock proteins in *S. typhimurium* (3). However, several experimental results make it difficult to formulate a simple model to relate these two phenomena. (i) Even though *xthA* mutants are highly sensitive to hydrogen peroxide, resistance to hydrogen peroxide lethality can be induced in these strains (6). (ii) The hydrogen peroxide-induced protein patterns of these mutants are basically the same as those of the wild type (unpublished data). (iii) *htpR* mutants, which are defective in induction of the synthesis of heat-shock proteins even at mild heat shock, are not more hydrogen peroxide sensitive than their *htpR*$^+$ parents (data not shown). Thus both phenotypes of *xthA* mutants could be independent effects of the limiting amount of the *xthA* gene product found in the mutant cells. Still, we cannot rule out more complicated models which would connect these two phenomena.

The post-heat shock recovery to normal protein synthesis after return to the lower temperature was also slower in *xthA* mutants compared with the wild type (unpublished data). This may help explain the observation that the *xthA* mutants are not very heat sensitive compared with *xthA*$^+$ strains as judged by their susceptibility to killing by a 50°C treatment (data not shown). If *xthA* mutants are just slower to respond after returning to lower temperature, one might expect the cells to recover eventually unless some irreversible process has taken place.

ACKNOWLEDGMENTS

We thank C. Georgopoulos and B. Weiss for providing strains, L. M. Marsh and J. H. Krueger for valuable discussions, and M. White for help in preparation of this manuscript.

This work was supported by Public Health Service grant GM28988 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Bloch, P. L., T. A. Phillips, and F. C. Neidhardt.** 1980. Protein identifications on O'Farrell two-dimensional gels: locations of 81 *Escherichia coli* proteins. J. Bacteriol. **141**:1409–1420.
2. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
3. **Christman, M. F., R. W. Morgan, F. S. Jacobson, and B. N. Ames.** 1985. Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in *Salmonella typhimurium*. Cell **41**:753–762.
4. **Cohen, S. N., and A. C. Y. Chang.** 1977. Revised interpretation of the origin of the pSC101 plasmid. J. Bacteriol. **132**:734–737.
5. **Cooper, S., and T. Ruettinger.** 1975. A temperature-sensitive nonsense mutation affecting the synthesis of a major protein of *Escherichia coli*. Mol. Gen. Genet. **139**:167–176.
6. **Demple, B., and J. Halbrook.** 1983. Inducible repair of oxidative DNA damage in *Escherichia coli*. Nature (London) **304**:456–458.
7. **Demple, B., J. Halbrook, and S. Linn.** 1983. *Escherichia coli xth* mutants are hypersensitive to hydrogen peroxide. J. Bacteriol. **153**:1079–1082.
8. **Demple, B., and S. Linn.** 1982. 5,6-saturated thymine lesions in DNA: production by ultraviolet light or hydrogen peroxide. Nucleic Acids Res. **10**:3781–3789.
9. **Elledge, S. J., and G. C. Walker.** 1983. Proteins required for ultraviolet light and chemical mutagenesis: identification of the products of the *umuC* locus of *Escherichia coli*. J. Mol. Biol. **164**:175–192.
10. **Goff, S. A., L. P. Casson, and A. L. Goldberg.** 1984. Heat shock regulatory gene *htpR* influences rates of protein degradation and expression of the *lon* gene in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81**:6647–6651.
11. **Grossman, A. D., J. W. Erickson, and C. A. Gross.** 1984. The *htpR* gene product of *E. coli* is a sigma factor for heat-shock promoters. Cell **38**:383–390.
12. **Krueger, J. H., and G. C. Walker.** 1984. *groEL* and *dnaK* genes of *Escherichia coli* are induced by UV irradiation and nalidixic acid in an *htpR*$^+$ -dependent fashion. Proc. Natl. Acad. Sci. USA **81**:1499–1503.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Lee, P. C., B. R. Bochner, and B. N. Ames.** 1983. AppppA, heat-shock stress and cell division. Proc. Natl. Acad. Sci. USA **80**:7496–7500.
15. **Mandel, M., and A. Higa.** 1970. Calcium-dependent bacteriophage DNA infection. J. Mol. Biol. **53**:159–162.
16. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1980. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Milcarek, C., and B. Weiss.** 1972. Mutants of *E. coli* with altered deoxyribonucleases. I. Isolation and characterization of mutants for exonuclease III. J. Mol. Biol. **68**:303–318.
18. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Neidhardt, F. C., R. A. Van Bogelen, and E. T. Lau.** 1983. Molecular cloning and expression of a gene that controls the high-temperature regulon of *Escherichia coli*. J. Bacteriol. **153**:597–603.
20. **Neidhardt, F. C., R. A. Van Bogelen, and V. Vaughn.** 1984. Genetics and regulation of heat-shock proteins. Annu. Rev. Genet. **18**:295–329.
21. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250**:4007–4021.
22. **Rogers, S., and B. Weiss.** 1980. *Escherichia coli* exonuclease III. Methods Enzymol. **65**:201–211.
23. **Rogers, S. G., and B. Weiss.** 1980. Cloning of the exonuclease III gene of *Escherichia coli*. Gene **11**:187–195.
24. **Sancar, A., R. P. Wharton, S. Seltzer, B. M. Kacinski, N. D. Clarke, and W. D. Rupp.** 1981. Identification of the *uvrA* gene product. J. Mol. Biol. **148**:45–62.
25. **Schlesinger, M. J., M. Ashburner, and A. Tissieres.** 1982. Heat shock from bacteria to man. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
26. **Tilly, K., N. McKittrick, M. Zylicz, and C. Georgopoulos.** 1983. The *dnaK* protein modulates the heat-shock response of *E. coli*. Cell **34**:641–646.
27. **Tobe, T., K. Ito, and T. Yura.** 1984. Isolation and physical mapping of temperature-sensitive mutants defective in heat-shock induction of proteins in *Escherichia coli*. Mol. Gen. Genet. **195**:10–16.
28. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage on *Escherichia coli*. Microbiol. Rev. **48**:60–93.
29. **Yamamori, T., K. Ito, Y. Nakamura, and T. Yura.** 1978. Transient regulation of protein synthesis in *Escherichia coli* upon a shift-up of growth temperature. J. Bacteriol. **134**: 1133–1140.