JOURNAL OF BACTERIOLOGY, Feb. 1990, p. 1151–1154
0021-9193/90/021151-04$02.00/0

Vol. 172, No. 2

Loss of 4.5S RNA Induces the Heat Shock Response and Lambda Prophage in *Escherichia coli*

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Received 2 June 1989/Accepted 13 November 1989

During depletion of 4.5S RNA, cells of *Escherichia coli* displayed a heat shock response that was simultaneous with the first detectable effect on ribosome function and before major effects on cell growth. Either 4.5S RNA is involved directly in regulating the heat shock response, or this particular impairment of protein synthesis uniquely induces the heat shock response. Several hours later, lambda prophage was induced and the cells lysed.

The 4.5S RNA of *Escherichia coli* is a small, stable RNA present in an amount roughly equal to that of a single tRNA species, corresponding to about 10,000 molecules per cell (10, 11, 14). Synthesis of 4.5S RNA is under stringent control, and its maturation includes cleavage by RNase P, a tRNA-processing enzyme (3, 12, 13). Genetic disruption and complementation analyses have shown that the gene for 4.5S RNA (*ffs*) is essential for cell growth (7).

Extracts prepared from cells depleted of 4.5S RNA are impaired in the ability to translate natural mRNA [although they can carry out poly(U)-directed poly-phenylalanine synthesis] (4). Suppressors of *ffs* map in the gene for elongation factor G and in the aminoacyl-tRNA synthetases for glutamate and valine (5, 6). In mutants carrying these suppressors much of the 4.5S RNA sediments with the 70S ribosome (5). Together these results suggest that the essential function of 4.5S RNA is ribosome based.

To determine the global effect of depletion of 4.5S RNA on the synthesis of individual proteins, we employed cells of strain FF239 (similar to the strain described in reference 7, in which the only copy of *ffs* is fused to the pTac promoter on a lambda prophage). Cultures were grown in glucose-rich MOPS (morpholinepropanesulfonic acid) medium (15, 21) without methionine and with IPTG (isopropyl-β-D-thiogalactopyranoside) (5 μM). Figure 1 shows the growth with IPTG and after removal of IPTG from the medium to halt synthesis of 4.5S RNA. Cultures were maintained at an optical density (420 nm) of between 0.1 and 1.0 by subculturing into fresh medium. The cell growth rate began to decrease approximately 4.5 h after removal of IPTG, corresponding to approximately a 50-fold increase in cell mass. Growth continued at a progressively decreasing rate until another threefold increase in mass had occurred, after which cell growth stopped and lysis occurred.

At the times indicated in Fig. 1, portions (1 ml) of the culture were labeled (for 5 min with [^{35}S]methionine [1,150 Ci/mmol, 50 μCi/ml]) and chased with cold methionine (for 3 min with 0.16 ml of 0.2 M methionine). Extracts and two-dimensional gels were prepared as described previously (2, 17).

Up to 2.5 h after the removal of IPTG, no qualitative differences were observed between the protein patterns in the control and the depleted cultures (data not shown). However, 3.5 h after removal of inducer, the rates of

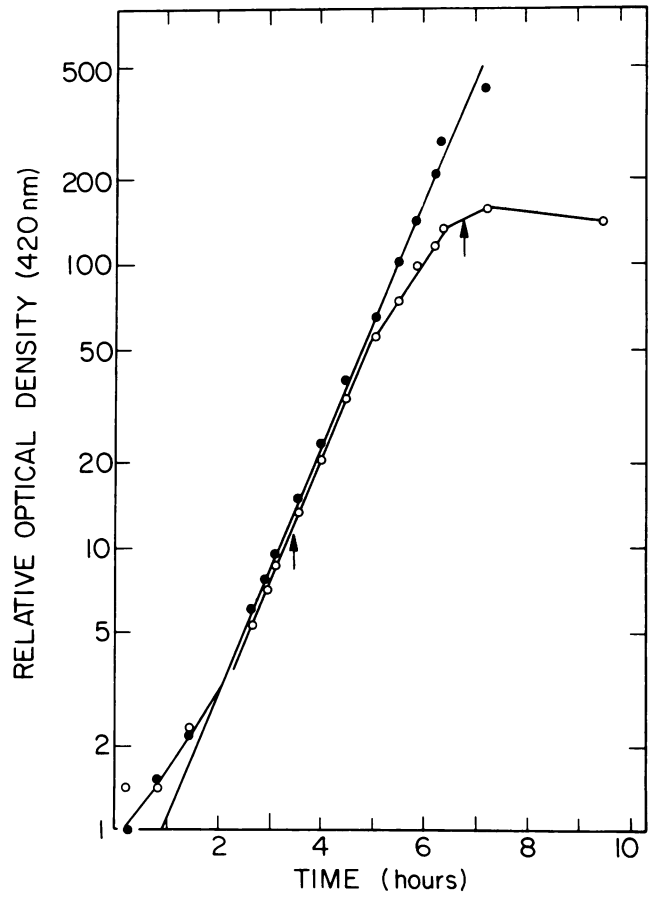

FIG. 1. Growth of *E. coli* FF239. Cultures of IPTG-dependent cells were grown with (●) or without (○) IPTG. The ordinate is expressed in relative optical density units to permit a continuous plot of growth of the culture, which was diluted at times to avoid high density. The arrows indicate the times at which samples were labeled for analysis on two-dimensional gels (Fig. 2).

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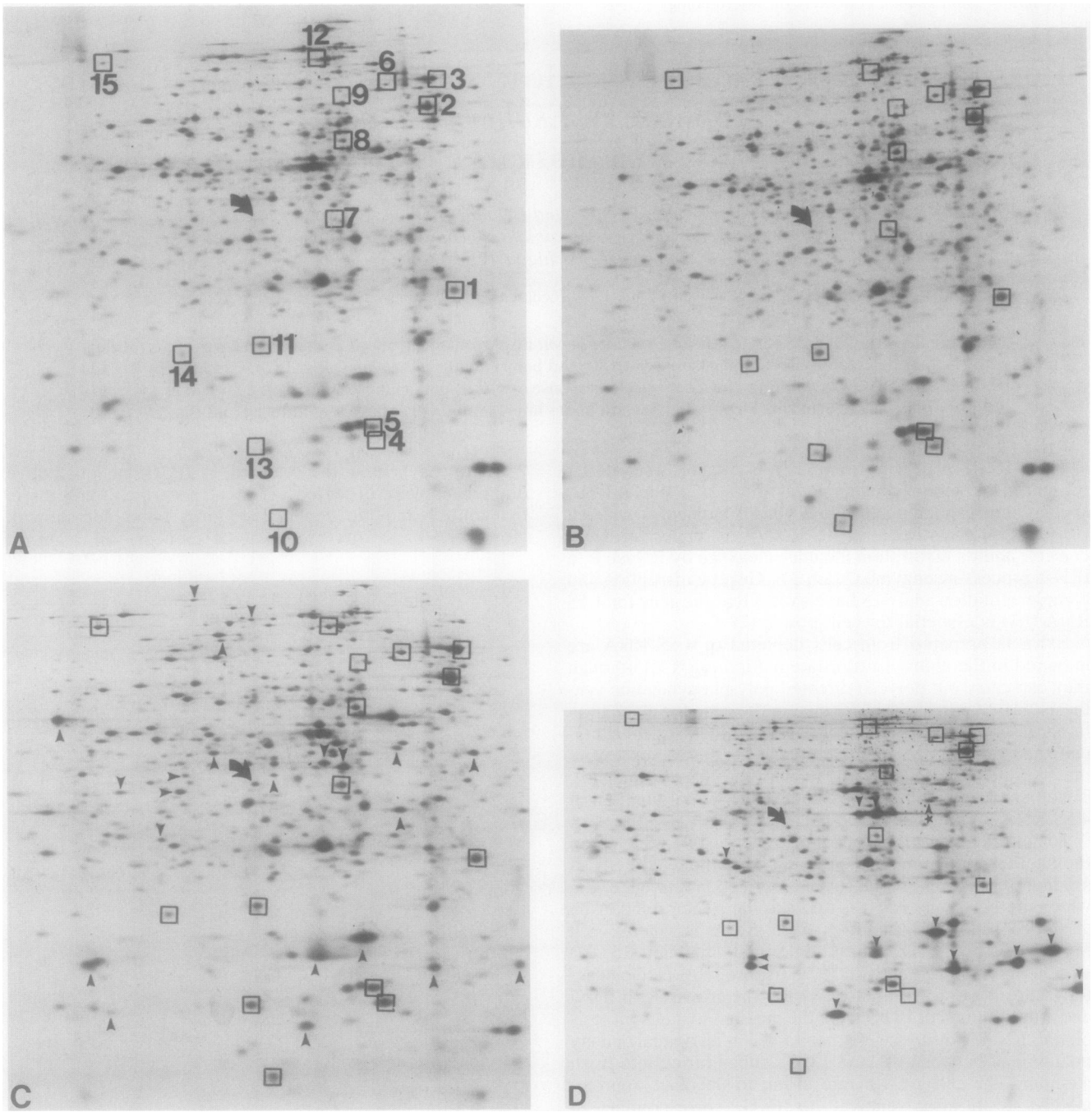

FIG. 2. Synthesis of individual proteins in the presence or absence of inducer IPTG. The autoradiograms show the patterns of proteins synthesized in the presence of IPTG (A), at 3.5 h (B) or 6.8 h (C) after removal of IPTG, or 30 min after a shift to 42°C with IPTG (D). The boxes surround heat shock proteins, numbered as follows: 1, B25.3; 2, B56.5; 3, B66.0; 4, C14.7; 5, C15.4; 6, C62.5; 7, D33.4; 8, D48.5; 9, D60.5; 10, F10.1; 11, F21.5; 12, F84.1; 13, G13.5; 14, G21.0; 15, H94.0. The large curved arrow points to sigma-32, the arrowheads in panel C indicate proteins induced 6.8 h after removal of inducer, and the arrowheads in panel D indicate proteins induced by induction of the prophage. RecA is indicated by a star next to the arrowhead in panel D.

synthesis of 16 proteins previously identified as sigma-32-dependent heat shock proteins (16) increased. These 16 proteins were the only heat shock proteins observable on equilibrium gels after a shift to 42°C (Fig. 2A and B). The response differed from that following a temperature shift-up only in that it continued unabated for several hours. No other proteins were markedly induced or repressed.

Shortly after cell growth stopped (6.8 h after removal of inducer, corresponding to a 150-fold increase in cell mass), major changes in the pattern of proteins synthesized were seen (Fig. 2C). These changes included hyperinduction of the heat shock proteins at rates equivalent to the peak rates of synthesis observed after a shift to 42°C. Additionally, approximately 22 more proteins (Fig. 2C, arrowheads) were

induced, and a similar number were repressed. Translation of the remaining 500 proteins was maintained at normal levels, despite the fact that cell viability had dropped significantly by this time (results not shown). Some of these 22 additional proteins could be shown to be the product of lambda prophage induced in these cells at the later times. A culture was grown with IPTG and shifted to 42°C to inactivate the lambda *c*I857 repressor. A portion (1 ml) of the culture was labeled with [^{35}S]methionine (1150 Ci/mmol, 50 μCi/ml) 30 to 40 min after the shift to 42°C. Extracts were prepared and analyzed on two-dimensional gels. The results (Fig. 2D) indicate that many of the unique proteins made after induction of the prophage by the temperature shift correspond to proteins made late after IPTG withdrawal (cf. Fig. 2C). The cause of prophage induction is not known. It might be related to the cessation of DNA synthesis in the cells at this time (4) or to degradation of the *c*I857 repressor made by this prophage, coupled with an inadequate rate of resynthesis in the inhibited cells.

Many inducers of the heat shock response have been reported (reviewed in reference 16). Next to a temperature shift, the best inducer of the heat shock response known until now was treatment with ethanol. Most other agents induce only a portion of the heat shock proteins, and their induction does not constitute the major cellular response to these agents (20). Here we report that depletion of 4.5S RNA induces a heat shock response indistinguishable from that of a temperature shift, save for its prolonged (>3-h) duration.

How does the depletion of 4.5S RNA elicit a heat shock response? Induction of the heat shock response in the depleted cells was accompanied by an elevation of the sigma-32 level (Fig. 2), as normally occurs after a shift to high temperature (18, 19). It is difficult to imagine how 4.5S RNA itself could directly modulate the heat shock response, since the elevation of sigma-32 levels appears to be the result largely of posttranscriptional regulation (18, 19).

A more plausible alternative is that the heat shock response is triggered by the translational block that develops in 4.5S RNA-depleted cells. Many inducers of the heat shock response (amino acid analogs, puromycin treatment, ethanol) are known to disrupt protein synthesis, and many others (UV irradiation, nalidixic acid, heavy metals, oxidants) could certainly do so indirectly. A temperature shift itself brings about accumulation of guanosine tetraphosphate and a transient, stringent shutdown of stable RNA synthesis (reviewed in reference 8). We see no unusual stuttering of protein spots in gels made from cells depleted of 4.5S RNA (Fig. 2), so there is no evidence that abnormal protein is accumulating to induce the heat shock response in the manner proposed by Goff and Goldberg (9). Rather, it would seem that the translational block generates an alarm signal for heat shock.

We cannot rule out the possibility that depletion of 4.5S RNA induces a heat shock response and prophage by disrupting some cellular process other than translation. But, since mutations in elongation factor G and some aminoacyl-tRNA synthetases can suppress the effects of 4.5S RNA depletion (5, 6), it would appear that the main function of 4.5S RNA is in some part of the translation process. For this reason the simplest hypothesis is that a translational defect triggers first the heat shock response and later the induction of lambda. Prophage induction could conceivably contribute to the prolongation of the heat shock response by elevating the sigma-32 level by a mechanism previously described (1).

Given the apparent authenticity of the heat shock response made by 4.5S RNA-depleted cells and the demonstrated alteration in their translational capacity, further studies to examine the relation between ribosome function and the heat shock response seem in order.

This work was supported in part by Public Health Service grants GM19351 (to M.J.F.) and GM17892 (to F.C.N.) from the National Institute of General Medical Sciences.

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