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FIS-Dependent *trans* Activation of Stable RNA Operons of *Escherichia coli* under Various Growth Conditions

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In *Escherichia coli* transcription of the tRNA operon *thrU* (*tufB*) and the rRNA operon *rrnB* is *trans*-activated by the protein FIS. This protein, which stimulates the inversion of various viral DNA segments, binds specifically to a *cis*-acting sequence (designated UAS) upstream of the promoter of *thrU* (*tufB*) and the P1 promoter of the *rrnB* operon. There are indications that this type of regulation is representative for the regulation of more stable RNA operons. In the present investigation we have studied UAS-dependent transcription activation of the *thrU* (*tufB*) operon in the presence and absence of FIS during a normal bacterial growth cycle and after a nutritional shift-up. In early log phase the expression of the operon rises steeply in wild-type cells, whereafter it declines. Concomitantly, a peak of the cellular FIS concentration is observed. Cells in the stationary phase are depleted of FIS. The rather abrupt increase of transcription activation depends on the nutritional quality of the medium. It is not seen in minimal medium. After a shift from minimal to rich medium, a peak of transcription activation and of FIS concentration is measured. This peak gets higher as the medium gets more strongly enriched. We conclude that a correlation between changes of the UAS-dependent activation of the *thrU* (*tufB*) operon and changes of the cellular FIS concentration under a variety of experimental conditions exists. This correlation strongly suggests that the production of FIS responds to environmental signals, thereby *trans*-activating the operon. Cells unable to produce FIS (*fis* cells) also show an increase of operon transcription in the early log phase and after a nutritional shift-up, albeit less pronounced than that of wild-type cells. Presumably it is controlled by the ribosome feedback regulatory system. *cis* activation of the operon by the upstream activator sequence is apparent in the absence of FIS. This activation is constant throughout the entire growth cycle and is independent of nutritional factors. The well-known growth rate-dependent control, displayed by exponentially growing cells studied under various nutritional conditions, is governed by two regulatory mechanisms: repression, presumably by ribosome feedback inhibition, and stimulation by *trans* activation. FIS allows very fast bacterial growth.

The synthesis of rRNA of *Escherichia coli* is finely tuned to the cell's environmental conditions. Cells growing in a constant environment do not show a significant turnover or a significant buildup of free rRNA or vacant ribosomes, except at very low growth rates (for reviews, see references 20, 21, and 26). Consequently, ribosomes are utilized at maximal or near-maximal capacity. Upon alteration of the nutritional capacity of the medium, leading to a different but constant environment, cells promptly readjust the synthesis of their rRNA and tRNA to meet the demands of an altered growth rate. In exponentially growing cells the concentration of ribosomes (and of rRNA) thus appears to be proportional to growth rate (6, 8, 9). The mechanism underlying this so-called growth rate-dependent control has been described as a feedback inhibition of rRNA synthesis by ribosomes (ribosome feedback control) (5, 12, 13, 29) and/or inhibition by ppGpp (stringent control) (4), the concentration of this nucleotide being a function of the growth medium (28). Gaal and Gourse reported that *E. coli* mutants unable to produce ppGpp are still under growth rate-dependent control. They concluded that ppGpp is not the only factor involved in this type of regulation (7).

Interestingly, it has been reported that the synthesis of tRNA is subject to the same regulatory mechanisms as the synthesis of rRNA (14, 32). While studying the regulation of the tRNA operon *thrU* (*tufB*), we recently showed that it is also regulated by a positively acting control system. Upstream of this operon a *cis*-acting sequence is found, deletion of which results in an 80 to 90% drop of transcription (31). This sequence, called UAS for upstream activator sequence, appeared to be the target of a *trans*-activating protein (34), which we subsequently identified as the protein FIS (3, 25). Up till then this heat-stable protein was only known to be involved in site-specific recombination (2, 17, 30). It stimulates the inversion of various viral DNA segments by binding to a recombinational enhancer (16, 19).

It may be envisaged that more stable RNA operons are activated in *trans* by this system. Accordingly, the UASs of the *tyrT*, *metY*, *thrU* (*tufB*), and *rrnB* operons all bind one and the same protein (25, 33). Sequences upstream of the P1 promoters of all rRNA operons and the promoters of 13 tRNA operons (but not all tRNA operons) match the consensus sequence for FIS-binding sites (18, 33). Recently Ross et al. (18, 27) independently demonstrated that FIS acts as the *trans* activator of the *rrnB* operon. In this context the question of under which conditions the *trans* activation control system becomes operative arises. Preliminary studies from this laboratory (3, 25) revealed large fluctuations in the *trans* activation of the *thrU* (*tufB*) operon during a normal bacterial growth cycle. Here we have studied these

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TABLE 1. *E. coli* strains used in this investigation

Strain	Genotype or phenotype	Reference or source
MC1000	<i>lacX74 araD139 (ara leu)7697 galU galK Str^r</i>	15
MC1000- <i>fis</i> 767	<i>lacX74 araD139 (ara leu)7697 galU galK Str^r fis Kan^r</i>	15
JM101	<i>supE thi (lac-proAB)</i>	22
JM101- <i>fis</i> 767	<i>supE thi (lac-proAB) fis Kan^r</i>	Our laboratory

fluctuations in more detail. We also asked whether changes in the level of *trans* activation occur in response to environmental signals and whether they are accompanied by changes in the cellular FIS concentration. Of further interest was whether growth rate-dependent control is solely governed by ribosome feedback (see above) or whether *trans* activation also plays a role in this process. Finally we describe the benefits for the cell of having FIS.

MATERIALS AND METHODS

Strains, plasmids, and growth media. The *E. coli* strains used in this study are listed in Table 1. The plasmid pDS10 is described in reference 31. It harbors the operon fusion *thrU (tufB)'*:*galK*, and the UAS extends from position -176 to the promoter. pDS10ΔUAS is identical to pDS10 except that it carries a deletion extending from -500 to -57.

M9 minimal medium was supplemented with thiamine (1 μg/ml), essential amino acids (20 μg/ml), and succinate (1.0%) (30); in the case of minimal medium plus amino acids supplementation was with 0.5% casein hydrolysate. LB medium (per liter: 10 g of Bacto Tryptone, 5 g of Bacto Yeast Extract, 10 g of NaCl, pH 7.5) was prepared by the method of Miller (23). In the nutritional shift-up experiment 0.1 volume of a 10× concentrated LB medium without NaCl was added. Brain heart infusion medium was prepared according to the manufacturer's description (Difco Laboratories, Detroit, Mich.). In most of the experiments with a rich medium the medium was replaced by LB plus glucose (1%), since this improved the reproducibility of the results.

Determination of galactokinase activity. The bacteria transformed with pDS10 or pDSΔUAS were grown overnight in the medium indicated. The cultures were diluted and incubated further at 37°C. At the times indicated the optical density at 600 nm (OD₆₀₀) was measured and samples were withdrawn. Galactokinase activities and plasmid copy numbers were determined by the method of Adams and Hatfield (1) with modifications by van Delft et al. (31).

Bacterial growth. Growth was measured by reading OD₆₀₀ (in most cases) or by determination of the dry weight of the biomass (see Fig. 8). In the latter case cells were sedimented and washed with 0.83% NaCl, whereafter the sediment was weighed after lyophilization.

Determination of the cellular FIS concentration. For each determination cell extracts were prepared from two independent bacterial cultures (40 h in minimal medium prior to the shift-up and overnight prior to a growth cycle in LB medium plus 1% glucose). After sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and Western immunoblotting with FIS antibodies pretreated with an extract of the *fis* strain MC1000-*fis*767, FIS was detected with the ECL fluorescence kit of Amersham. Fluorescence signals were quantified by determining peak areas with a laser scanner. These areas were correlated with the area of a cross-reacting protein representative for the protein concentration in the samples. The relative FIS concentration plot-

ted in the figures represents the indirect FIS concentration/total soluble protein. It is corrected for blot and detection efficiency by using purified FIS as an internal standard. The standard deviation was calculated for each point with the data of three independent Western blots and two scans of each blot. The largest deviation found was 15%.

RESULTS

Experimental strategy. In order to study the expression of the *thrU (tufB)* operon in vivo under various cellular growth conditions we used the plasmid-borne operon fusion as previously described (31). This fusion, *thrU (tufB)'*:*galK*, which puts the expression of *galK* under control of the *thrU (tufB)* promoter, was introduced into *galK*-defective *E. coli* strains. Expression of the operon was studied by measuring galactokinase activity/femtomole of plasmid. Inherent to this procedure is that a difference in life span of the galactokinase protein and the transcript may lead to an overestimation of the number of transcripts present at the times indicated and that galactokinase activities lag somewhat behind changes in FIS concentration and transcription. When relevant, this is pointed out in the text (e.g., see Fig. 3A). Large fluctuations in galactokinase activity are observed during a bacterial growth cycle and after a nutritional shift-up (cf. Fig. 2 to 4). These are due to synthesis and degradation of the galactokinase protein, since they cannot be ascribed to comparable fluctuations in plasmid copy numbers. Changes in the rate of galactokinase mRNA or protein degradation, occurring under the various growth conditions, may thus bias the results. This drawback can be overcome as indicated below.

Since transcription activation of the operon depends on the presence of the UAS, galactokinase activities were measured in cells carrying a plasmid with an intact UAS (designated UAS⁺ cells) or with the UAS deleted (designated UAS⁻ cells). Other regulatory mechanisms, known to control stable RNA synthesis such as ribosome feedback inhibition and stringent response, have DNA determinants in the region from position -50 to +1 (11). DNA determinants of transcription activation, however, are found in the region extending from -131 to -48 (25, 31, 34). This enabled us to distinguish between the effect of UAS-dependent activation and that of repression by another regulatory mechanism(s). Galactokinase activities of UAS⁺ cells thus reflect the effects of all regulatory mechanisms including activation, whereas the activities of UAS⁻ cells reflect the effects of all mechanisms except activation. The ratio of galactokinase activities of UAS⁺ and UAS⁻ cells is a measure of activation. It is independent of changes in the rate of mRNA or protein degradation.

We have also studied the effect of FIS on activation and on other regulatory systems by performing the same experiments in a strain lacking a functional *fis* gene (*fis* cells). The complete lack of FIS in *fis* cells was confirmed by Western blotting with FIS-specific antibodies (data not shown).

Expression of the *thrU (tufB)* operon during a bacterial

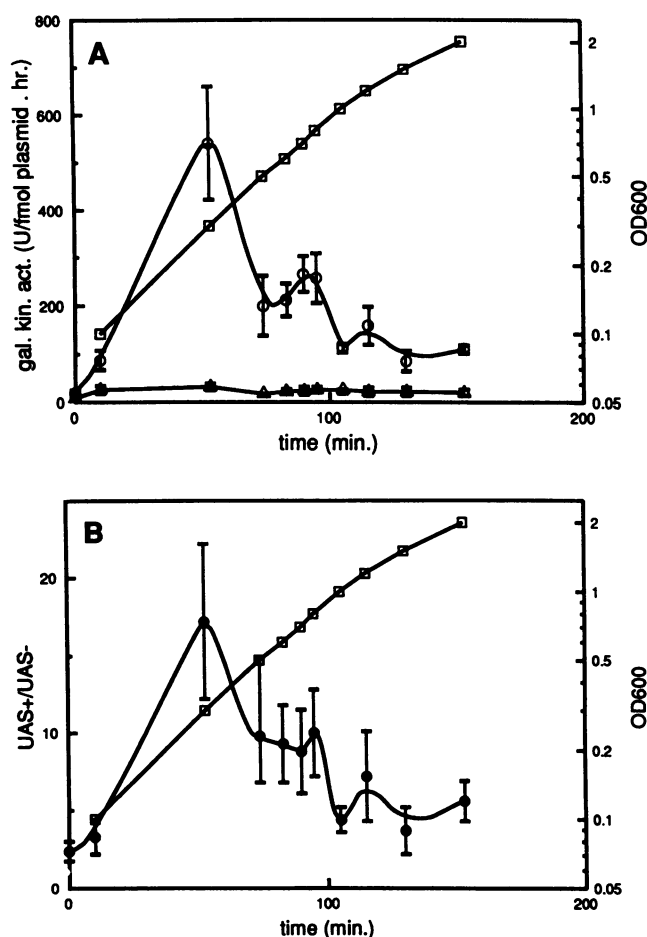


FIG. 1. Expression of the plasmid-borne *thrU* (*tufB*) operon during a bacterial growth cycle. *E. coli* MC1000 was transformed with pDS10 or the plasmid deletion derivative lacking the UAS, pDS10ΔUAS (see Materials and Methods). The cells were grown overnight in brain heart infusion medium, diluted to an OD₆₀₀ of 0.1, and incubated at 37°C. (A) Galactokinase activities expressed in units per femtomole of plasmid per hour. ○, UAS⁺ cells; △, UAS⁻ cells; □, OD₆₀₀. (B) Ratios of galactokinase activities in UAS⁺ and UAS⁻ cells (●) as calculated from the data in panel A. Standard errors (SE) of the ratios were determined by the following formula: $[(SE \text{ of } UAS^+ \text{ activity}/UAS^+ \text{ activity})^2 + (SE \text{ of } UAS^- \text{ activity}/UAS^- \text{ activity})^2]^{0.5}$. The error bars represent the SE, in each case based on three independent experiments. □, OD₆₀₀. Background levels of galactokinase activities, representing nonspecific binding of radioactive material to the membrane and enzymatic activity at 0°C, were $7\% \pm 2\%$ for UAS⁺ cells and $6\% \pm 4\%$ for UAS⁻ cells.

growth cycle. With the strategy outlined above the expression of the *thrU* (*tufB*) operon was investigated during a normal bacterial growth cycle in various media. Overnight cultures were diluted to an OD₆₀₀ of 0.1, and galactokinase activities were measured at various stages thereafter. Figure 1A shows the results obtained with cells grown in a rich medium (brain heart infusion). Large fluctuations in galactokinase levels are seen during the growth cycle, which is in agreement with our previous results (25). Almost immediately after dilution of a stationary phase culture in fresh medium, a large increase of galactokinase activity occurs in UAS⁺ cells. That this increase is mainly due to activation of transcription can be concluded from the behavior of UAS⁻ cells. The latter cells also show an increase in galactokinase

activity, but it is much less pronounced. Apparently, an additional regulatory mechanism differing from activation becomes operative after reinitiation of growth but the stimulation of transcription by this mechanism is greatly outweighed by the activation of the operon. The increase in activation is clearly reflected by the ratio of galactokinase activities in UAS⁺ and UAS⁻ cells (Fig. 1B).

The initial rise in galactokinase activities is followed by a drop in both UAS⁺ and UAS⁻ cells. The decline in activation is reflected by a decrease of the ratio of galactokinase activities in UAS⁺ and UAS⁻ cells from 17 to approximately 10 (Fig. 1B). After this rather steep drop, activation declines further, albeit more slowly, as indicated by ratios of 4 to 5 when cells approach the stationary phase.

Sensing the nutritional quality of the medium. The increase in activation in early log phase is affected by the composition of the medium. This became apparent by studying the UAS⁺-to-UAS⁻ ratios during outgrowth of overnight cultures in various media. The ratio varies from less than 5 in minimal medium to approximately 16 in LB medium and 17 in brain heart infusion medium (maximal ratios are not always observed at the same cell concentration). Even though we cannot fully rule out that we have missed the exact activation peak during the growth cycle, the large differences observed indicate that the cells sense the nutritional quality of the medium and respond with an altered activation level.

Transcription regulation in the presence and absence of FIS. Transcription activation of the *thrU* (*tufB*) operon is dependent on the protein FIS (3, 25), as is clearly shown by comparing the expression of the operon in wild-type and *fis* cells (Fig. 2). Overnight cultures of both types of cells are devoid of FIS (see below). Accordingly, deletion of the UAS has the same effect on the galactokinase activities of stationary wild-type and *fis* cells, i.e., an approximately threefold reduction of the galactokinase activities (compare zero time activities in Fig. 2A and B). Apparently, we are dealing here with a *cis* effect of the UAS in the absence of FIS. This *cis* effect should be constant throughout the entire growth cycle in *fis* cells. This is indeed what the experiment shows, as can be concluded from the UAS⁺-to-UAS⁻ ratios (Fig. 2B and Table 2). A FIS-independent effect of the UAS on the expression of the *rrnB* operon has been shown, both in vivo and in vitro, by Gourse and coworkers (27). In contrast to what is seen in *fis* cells, the UAS⁺-to-UAS⁻ ratio rapidly increases in wild-type cells (Fig. 2A and Table 2). The activation that becomes operative in these cells thus is due to two effects: one is dependent on FIS occurring immediately after reinitiation of growth, and the other most likely is a FIS-independent *cis* effect induced by the nucleotide sequence, since it acts continuously and at a constant level.

Figure 2A and B further show expression of the *thrU* (*tufB*) operon after deletion of the UAS in wild-type and *fis* cells. As mentioned above, an additional control mechanism, different from UAS-dependent activation, becomes apparent here. We suggest that it reflects derepression of ribosome feedback inhibition control.

Interestingly, the UAS-independent transcription persists for a longer period of time in *fis* cells than in wild-type cells, leading to a significantly higher galactokinase level in *fis* cells than in wild-type cells. A similar phenomenon is observed with the *rrnB* operon (27). Although further experiments are needed, these data suggest that UAS-dependent activation and the additional regulatory system affect each other.

As can be seen in Table 2 and Fig. 2A and B, expression of the *thrU* (*tufB*) operon with an intact UAS is almost equal

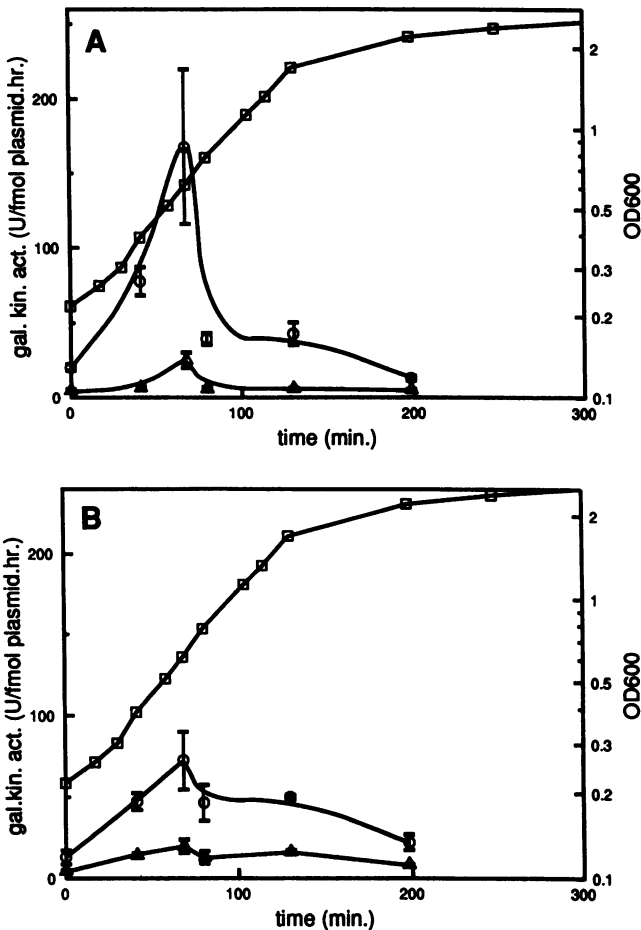


FIG. 2. Effects of FIS and UAS on *thrU* (*tufB*) expression during growth cycle. Wild-type cells (MC1000) (A) and *fis* cells (MC1000-*fis*767) (B) were transformed with the plasmids described in the legend to Fig. 1. Overnight cultures were diluted to an OD₆₀₀ of 0.2 in LB medium and grown at 37°C. Galactokinase activities are expressed as described in the legend to Fig. 1. For symbol definitions, see the legend to Fig. 1.

in wild-type and *fis* cells for a certain period of time (between OD₆₀₀ of 0.8 and 1.6). Since *fis* cells cannot use the transcription activation system, they apparently compensate for it by derepressing the ribosome feedback control system.

Cellular levels of FIS. The large fluctuations in transcrip-

TABLE 2. Galactokinase activity ratios of various cell types (MC1000 and MC1000-*fis*767) determined during the growth cycle^a

OD ₆₀₀	Galactokinase activity ratio of:		
	WT UAS ⁺ /WT UAS ⁻	WT UAS ⁺ / <i>fis</i> UAS ⁺	<i>fis</i> UAS ⁺ / <i>fis</i> UAS ⁻
0.2	4.1 ± 0.87	1.5 ± 0.52	2.9 ± 1.0
0.4	12.0 ± 2.9	1.6 ± 0.26	3.3 ± 0.45
0.6	6.7 ± 2.4	2.3 ± 0.91	3.6 ± 1.2
0.8	6.5 ± 1.2	0.84 ± 0.22	3.6 ± 1.3
1.6	6.8 ± 1.3	0.86 ± 0.16	3.0 ± 0.2
2.2	2.5 ± 0.32	0.59 ± 0.15	2.6 ± 0.55
2.4	3.5 ± 0.67	0.56 ± 0.12	3.8 ± 1.0

^a See legends to Fig. 2A for wild-type (WT) cells and Fig. 2B for *fis* cells.

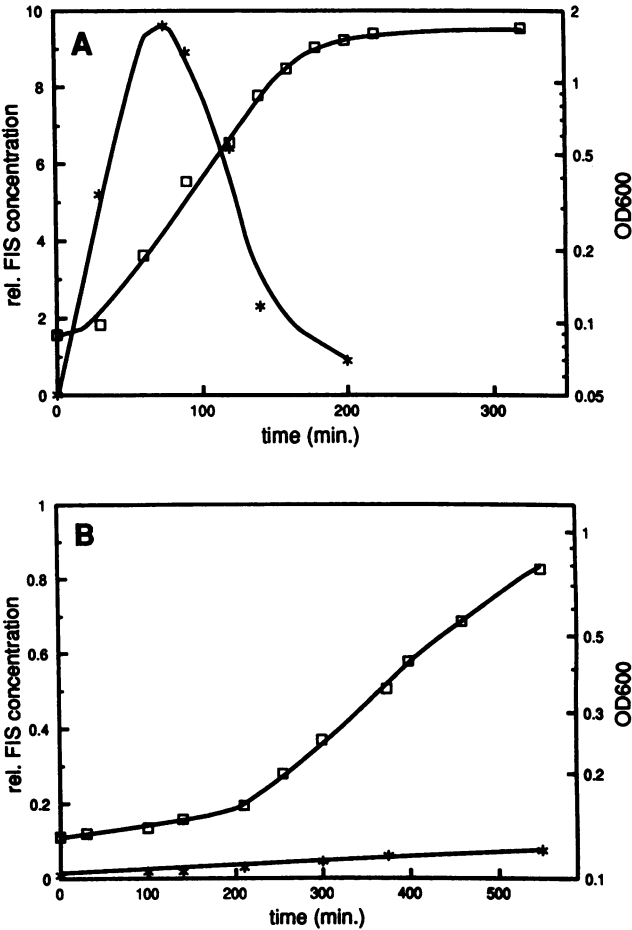


FIG. 3. Cellular FIS concentration during growth cycle. Overnight cultures of the wild-type strain MC1000 were diluted to an OD₆₀₀ of 0.15 and grown at 37°C in LB medium plus 1% glucose (A) or in minimal medium (1% succinate) (B). At the times indicated the cellular FIS concentration was determined as described in Materials and Methods. *, relative FIS concentration (see Materials and Methods); □, OD₆₀₀.

tion activation of the *thrU* (*tufB*) operon during the growth cycle raise the question of whether the cellular level of FIS also varies. Thompson et al., using a FIS-DNA binding assay, reported that the FIS level dropped 70-fold as cells went from late log to stationary phase (30). We determined the cellular FIS concentration during the entire growth cycle by using Western blotting. Cells cultured in a rich medium (LB plus 1% glucose) show a rapid increase of their FIS level in the early log phase (Fig. 3A). A peak value of FIS is reached approximately 75 min after initiation of the growth cycle, whereafter a rather steep decline sets in. In the stationary phase FIS has dropped to a level at which it is undetectable and so has transcription activation. The maximum level of FIS does not exactly coincide with that of galactokinase activity (cf. Fig. 2A and 3A). This is to be expected, since the galactokinase activities give an indirect estimate of the number of transcripts and will lag behind changes in transcription. Conceivably, the fluctuations in FIS level largely govern the fluctuations in transcription activation during the growth cycle in this medium.

Cells growing in minimal medium (1% succinate) do not show an abrupt rise in FIS content. The slight gradual

increase of FIS observed in Fig. 3B is at the lower limits of detection and may be of questionable significance. We conclude that the fluctuations in the cellular concentration of FIS are sensitive to environmental signals. Since transcription activation also varies with changes in the composition of the medium, a correlation between changes in the UAS-dependent transcription activation of the *thrU* (*tufB*) operon and changes of the cellular FIS concentration becomes apparent. This correlation strongly suggests that environmental conditions signal *trans* activation of the operon by FIS. Further confirmation of this correlation was obtained by studying the effect of a nutritional shift-up.

Effects of a nutritional shift-up. Figure 4 shows the response of exponentially growing wild-type and *fis* cells to a shift from minimal medium to LB medium. The growth rate of wild-type cells promptly increases from 0.4 to 2.2 doublings per h (not shown), while galactokinase activity increases approximately 10-fold over a period of 2 h after the shift (Fig. 4A). This enhanced expression of the *thrU* (*tufB*) operon is greatly due to a rise in transcription activation, since the UAS⁺-to-UAS⁻ ratio increases approximately fourfold during this period.

The cells also respond with a change in their FIS content (Fig. 4B). As illustrated above (Fig. 3B), cells growing in minimal medium do not display sudden fluctuations in FIS concentration during the growth cycle, and this concentration remains very low. Immediately after changing the composition of the medium, however, a steep rise of FIS occurs within a 1-h period, whereafter it also declines rather steeply and levels off at a relatively elevated level.

Cells unable to produce FIS (*fis* cells [Fig. 4C]) likewise sense this rather drastic change in the composition of the medium. The galactokinase activity rises, although not to the same level as in wild-type cells, but the UAS⁺-to-UAS⁻ ratio remains constant. Apparently, a regulatory mechanism, different from *trans* activation, is responsible for this enhancement of the *thrU* (*tufB*) expression.

A limited nutritional shift up from 1% succinate to 1% Casamino Acids plus glucose also enhances the growth rate of wild-type cells (from 0.4 to 1.1 doublings per h [not shown]) and the expression of the *thrU* (*tufB*) operon, as can be concluded from Fig. 5. Galactokinase activity rises over a period of about 1 h (Fig. 5A). That *trans* activation participates in the enhanced transcription can be concluded from the increases in the UAS⁺-to-UAS⁻ ratio and in cellular FIS level (Fig. 5A and B, respectively). Finally, *fis* cells also respond with an increase in galactokinase activity (Fig. 5C). Since *trans* activation does not take place in these cells (the UAS⁺-to-UAS⁻ ratio remains approximately constant), the elevated expression of the operon is governed by a different control mechanism, most likely ribosome feedback control. Apparently the responses of wild-type and *fis* cells to a limited nutritional shift-up are quantitatively rather similar but qualitatively different in the regulatory mechanism responsible for the change in transcription.

Pulse-chase experiments. Although FIS-dependent *trans* activation so far has only been demonstrated in vivo and in vitro for the *thrU* (*tufB*) and *rrnB* operons (3, 18, 25, 27), various data suggest that the regulation of these operons is representative for that of more stable RNA operons (see above). In this context one may ask whether the stimulation of stable RNA synthesis by a nutritional shift, as studied more directly by pulse-chase experiments, differs in wild-type and *fis* cells. A direct comparison of the results of Fig. 4 with those of a pulse-chase experiment in which minimal medium is shifted to LB medium is technically not feasible,

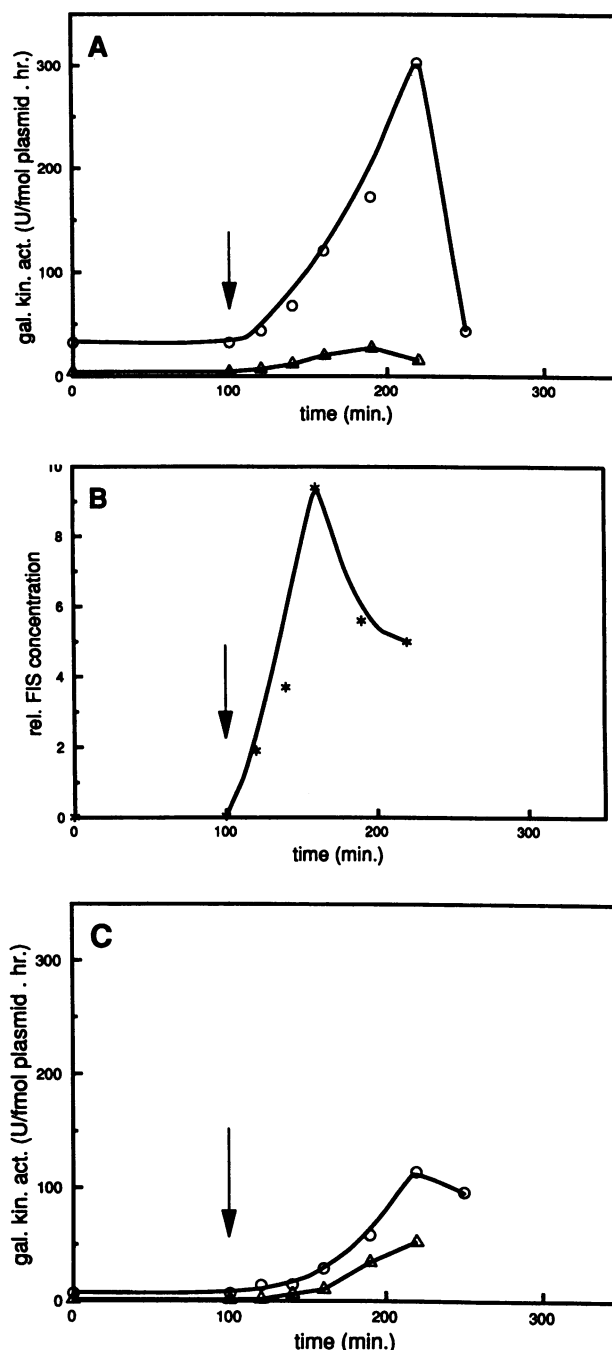


FIG. 4. Response to an extensive nutritional shift-up. Cells grown overnight in minimal medium were diluted 10-fold with minimal medium and grown at 37°C for 2 h, whereafter (arrow) the medium was shifted to LB medium plus 1% glucose. At the times indicated galactokinase activity and FIS concentration were determined as described in Materials and Methods. (A) Galactokinase activities of wild-type MC1000 cells transformed with pDS10 (○) (UAS⁺ cells) or with pDS10ΔUAS (Δ) (UAS⁻ cells); (B) relative cellular FIS concentration of MC1000 cells (*); (C) galactokinase activities of MC1000-*fis*767 (*fis* cells), transformed with pDS10 (○) (UAS⁺ cells) or pDS10ΔUAS (Δ) (UAS⁻ cells).

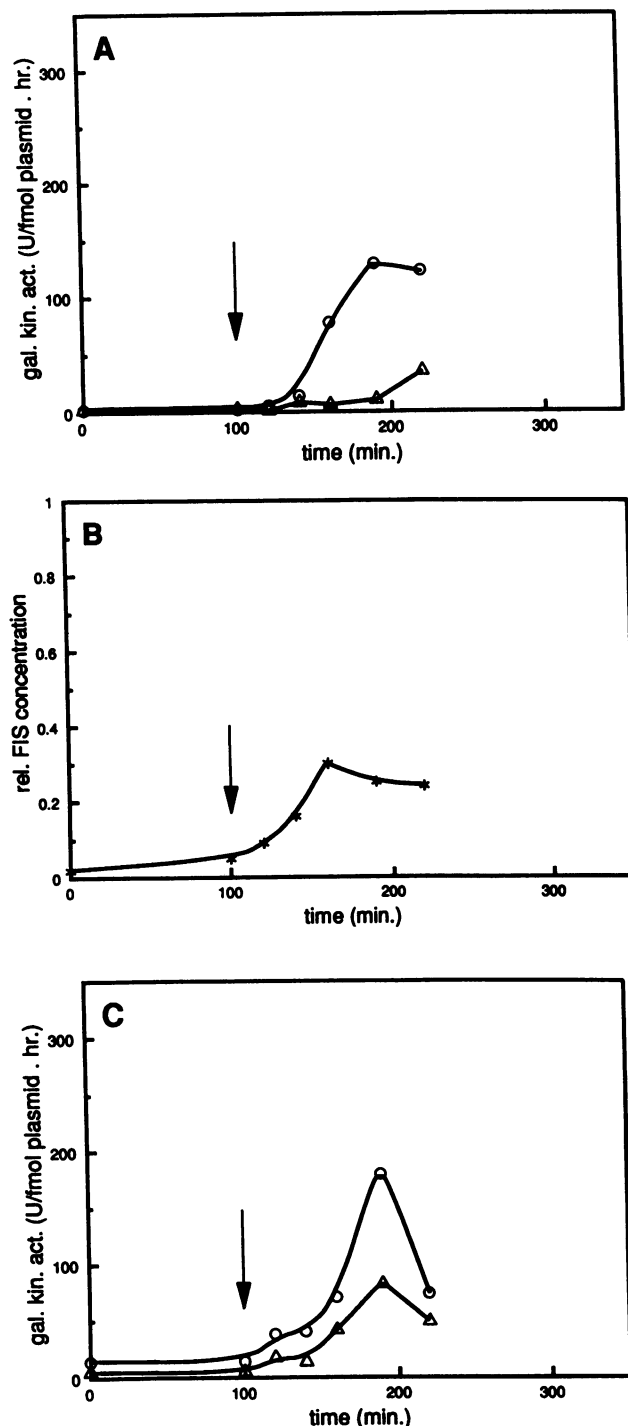


FIG. 5. Response to a limited nutritional shift-up. Experimental conditions were identical to those described in the legend to Fig. 4, except that the medium was shifted from minimal to Casamino Acids (1%) plus glucose (1%).

since the label is diluted out in LB medium. Shifting from 1% succinate to 1% Casamino Acids plus 1% glucose was therefore carried out instead. Cells received a 2-min [^3H]uridine pulse which was followed by an 8-min chase. The results differed somewhat depending on the pretreatment of the cells prior to the shift, but essentially the outcome was as illustrated in Fig. 6. Stable RNA synthesis in both wild-type

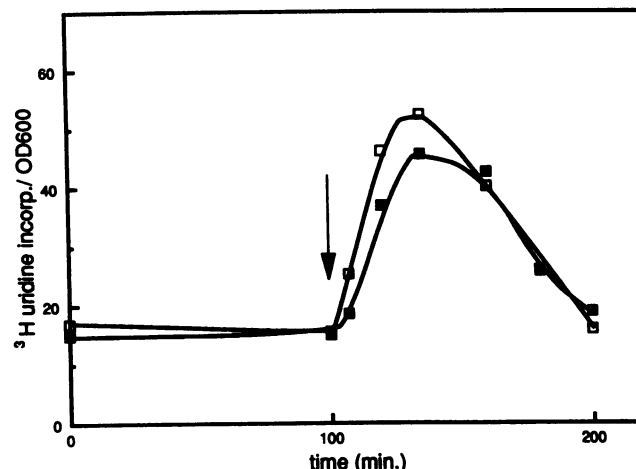


FIG. 6. Stable RNA synthesis after a limited nutritional shift-up, studied by pulse-chase experiments. MC1000-*fis*767 (*fis*) cells were cultured in minimal medium and shifted from minimal medium to Casamino Acids (1%) plus glucose (1%). One-milliliter culture samples were pulse-labeled with 37 KBq of [^3H]uridine for 2 min, which was followed by a chase with 0.125 mM nonlabeled uridine, whereafter 0.2 ml of a nonlabeled overnight culture as a carrier and 3 volumes of 7% trichloroacetic acid were added. Precipitates were collected on GF/C filters, washed, dried, and counted in a liquid scintillation counter ■, wild-type MC1000 cells; □, *fis* cells.

and *fis* cells increases immediately after the change of the medium. We conclude that the response of stable RNA synthesis to a limited nutritional shift is rather similar to that of *thrU* (*tufB*) expression. The results of Fig. 6 are also in line with those of Fig. 5 in that they do not reveal large quantitative differences in the responses of wild-type and *fis* cells. Qualitatively the responses of both cell types differ; however, since wild-type cells use *trans* activation to stimulate the expression of the *thrU* (*tufB*) operon (cf. Fig. 5B), whereas *fis* cells do not (see Discussion).

Growth rate-dependent *trans* activation. The fact that *rrn* operons lacking the UAS are submitted to growth rate-dependent control (11) indicates that cells incapable of *trans* activation utilize the mechanism of negative regulation by ribosome feedback. However, since the present investigation shows that the composition of the medium has a pronounced effect on the UAS-dependent *trans* activation, it does not seem very likely that growth rate-dependent regulation is solely governed by repression of stable RNA synthesis. In order to investigate this question we have grown UAS⁺ and UAS⁻ cells in different media, permitting a variation of the growth rates of between 0.9 and 2.0 doublings per h. Cells were harvested at an OD₆₀₀ of 0.4, and their galactokinase activities were determined. In this way the present results are fully comparable to those obtained by Gourse and coworkers (11), who studied the DNA determinants of growth rate-dependent control of the *rrnB* operon. As is apparent from Fig. 7, both UAS⁺ and UAS⁻ cells show a linear relationship between the galactokinase activities and the growth rates, in accordance with the findings for the *rrnB* operon (11). In this range of growth rates the increase in galactokinase activities is 2.6 times for UAS⁻ cells but 4.6 times for UAS⁺ cells. Increased growth rate thus leads to an increased UAS⁺-to-UAS⁻ ratio. We conclude that two control mechanisms underlie growth rate-dependent regulation: repression, presumably by ribosome feedback, and stimulation by *trans* activation.

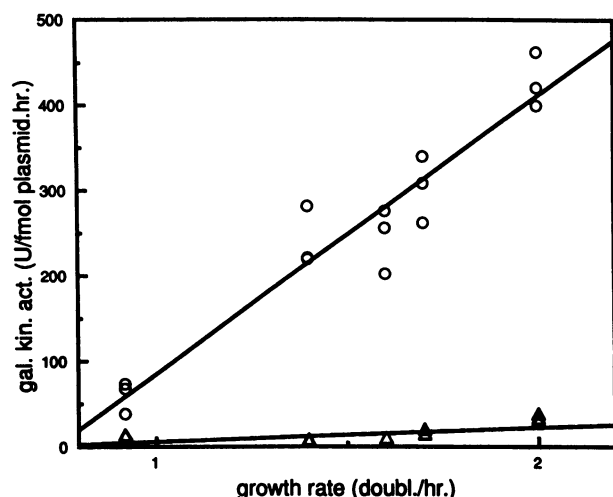


FIG. 7. Expression of the *thrU* (*tufB*) operon in exponentially growing cells at various growth rates. Transformants described in the legend to Fig. 1 were grown overnight in LB medium and diluted to an OD_{600} of 0.1 in different media. Growth rates were determined during the early log phase, and galactokinase activities were measured at an OD_{600} of 0.4. Background galactokinase levels were $6\% \pm 1.5\%$ for UAS^+ cells and $20\% \pm 4\%$ for UAS^- cells (cf. legend to Fig. 1). The media used were minimal medium, minimal medium supplemented with Casamino Acids, LB medium, LB medium supplemented with glucose, and brain heart infusion medium ($\circ$, UAS^+ cells; Δ , UAS^- cells).

FIS allows very fast cellular growth. We have seen that the expression of the *fis* gene is very pronounced when stationary cells reinitiate growth in a rich medium (Fig. 1 to 3). A rapid increase of FIS is accompanied by a rise of FIS-dependent *trans* activation of the *thrU* (*tufB*) operon. One may therefore expect wild-type cells to grow faster than *fis* cells under these conditions. This expectation is borne out by the following experiment. Wild-type (MC1000) cells and *fis* (MC1000-*fis*767) cells were grown in LB medium supplemented with 1% glucose to an OD_{600} of 0.3. The FIS concentration of wild-type cells has then reached its maximum (cf. Fig. 3A). At this stage both types of cells were diluted 40-fold, whereafter the OD_{600} was carefully monitored. At regular intervals samples were collected to determine the dry weight of the accumulated cell mass. As can be concluded from Fig. 8, growth rates of 2.2 and 1.4 doublings per h were found for wild-type and *fis* cells, respectively, on the basis of readings of OD_{600} , and growth rates of 1.8 and 1.3 doublings per h on the basis of biomass assays. The effect of the *fis* deletion is not strain specific, since we obtained the same result with the strain JM101.

Recently, Gille et al. (10) reported that FIS binds and bends the origin of chromosomal DNA replication, *oriC*, of *E. coli* and that FIS is required for minichromosome replication. The difference in growth rate of wild-type and *fis* cells, as measured here by reading OD and by assaying biomass, cannot be ascribed to a reduced replication ability. We conclude that exponentially growing cells able to *trans*-activate stable RNA synthesis have an advantage over cells unable to *trans*-activate and that one of the functions of FIS is to allow fast cellular growth (see Discussion).

DISCUSSION

Acceleration of cellular growth is accompanied by an elevated transcription of the *thrU* (*tufB*) operon. Such an

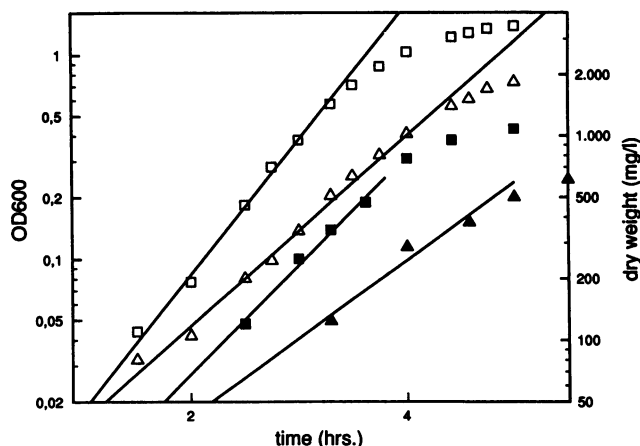


FIG. 8. Growth of wild-type cells (MC1000) and *fis* cells (MC1000-*fis*767) in rich medium. Cells grown to an OD_{600} of 0.3 in LB medium plus 1% glucose were diluted 40-fold and grown at 37°C in the same medium. At the times indicated, OD_{600} (open symbols) was read and the dry weight of the accumulated cell mass (closed symbols) was determined (see Materials and Methods). $\square$ and $\blacksquare$, wild-type cells; Δ and $\blacktriangle$, *fis* cells.

acceleration clearly occurs after dilution of an overnight culture in fresh medium (Fig. 1 and 2) and after a shift from minimal to rich medium (Fig. 4). In both cases the rise in transcription is dependent on the UAS, the target of the protein FIS. Deletion of the UAS abolishes the increase of *thrU* (*tufB*) expression in the early log phase almost completely (Fig. 1A). The ratio of galactokinase activities of cells carrying the plasmid-borne operon fusion *thrU* (*tufB*):*galK* with an intact or a deleted UAS (the UAS^+ -to- UAS^- ratio) rises substantially (Fig. 1B). As pointed out above (see "Experimental strategy" above), this ratio is a measure of transcription activation which is independent of changes in the rates of galactokinase mRNA and galactokinase protein degradation during the course of the experiment. In cells harboring the *fis* gene, this activation increases immediately after growth acceleration and in general declines thereafter. In cells unable to produce FIS (*fis* cells) the UAS^+ -to- UAS^- ratio remains constant throughout the entire growth cycle. This FIS-independent *cis* activation is not affected by environmental conditions. We assume that it is induced by the nucleotide sequence of the UAS. Increases of the UAS^+ -to- UAS^- ratio are also seen in wild-type cells after a nutritional shift-up (Fig. 4A and 5A). In contrast this ratio remains constant in *fis* cells (Fig. 4C). Concomitant with the changes in transcription activation, the cellular FIS concentration rises in wild-type cells, both in early log phase and after a shift-up (Fig. 3A, 4B, and 5B). Apparently, a correlation between changes of the UAS-dependent activation of the *thrU* (*tufB*) operon and changes of the cellular FIS concentration exists.

Various lines of evidence indicate that environmental conditions affect the FIS production and the activation of transcription. First, the UAS^+ -to- UAS^- ratios during bacterial outgrowth in fresh medium vary with the composition of the medium. The large rise and fall of FIS, observed during the growth cycle in a rich medium (Fig. 3A), are not observed during a cycle in minimal medium (Fig. 3B). After a shift from minimal to rich medium, however, both the cellular FIS content and operon transcription activation increase promptly (Fig. 4B and A, respectively). The in-

creases of FIS and of transcription activation are larger as the medium is more strongly enriched (cf. Fig. 4B and 5B).

We conclude that environmental conditions affect transcription activation and the FIS concentration in the same way. This correlation, apparently occurring under a variety of conditions (see above), strongly suggests that it is FIS that *trans*-activates transcription of the *thrU* (*tufB*) operon in *E. coli*. The alternative model, that FIS concentrations change in response to changes in stable RNA synthesis, is not a very logical one and has some rather bizarre implications. FIS-dependent *trans* activation apparently acts as a sensor of the nutritional quality of the medium. These data raise interesting questions concerning the regulation of de novo FIS synthesis and the transduction of the environmental signals involved.

As pointed out in the introduction, a number of observations indicate that the *trans* activation of the *thrU* (*tufB*) operon is representative for such a regulation of more stable RNA operons. One and the same protein binds in vitro to the UASs of the *tyrT*, *metY*, and *thrU* (*tufB*) operons and to the UAS upstream of the P1 promoter of the *rrnB* operon (25). FIS-dependent *trans* activation of the *rrnB* operon has been demonstrated by Ross et al. (18, 27). Sequence comparisons of upstream regions (18, 33) are also in line with the assumption that more stable RNA operons (albeit not necessarily all) are submitted to this regulatory system and respond to FIS. If so, this would indicate that one of the basic elements of growth control is the regulation of the de novo synthesis of FIS.

Cells unable to produce FIS (*fis* cells) also show enhanced transcriptional activity of the *thrU* (*tufB*) operon in early log phase of the growth cycle (Fig. 2B) and after enrichment of the medium (Fig. 4C and 5C), albeit in rich media to a lesser extent than wild-type cells. Since no FIS-dependent *trans* activation of the operon takes place in these cells, the UAS⁺-to-UAS⁻ ratio remains virtually constant, irrespective of the environmental conditions. The fact that galactokinase activity of UAS⁺ cells exceeds that of UAS⁻ cells reflects *cis* activation of the operon by the UAS in the absence of FIS. Significant transcription of the operon is thus observed in *fis* cells during the growth cycle (Fig. 2B). In fact, wild-type and *fis* cells only show a pronounced difference in UAS-dependent expression of the operon during the initial transcriptional jump in early log phase (cf. Fig. 2A and B). Thereafter, virtually no difference is seen for a certain period of time (between OD₆₀₀ of 0.8 and 1.6).

A possible explanation of these data is the following. In wild-type cells this leveling off at a lower level is due to ribosome feedback and a drop in FIS concentration. Ribosome feedback in these cells may result from a temporary excess of ribosomes, present after the steep rise in stable RNA synthesis in early log phase. In *fis* cells this rise is less pronounced and so may be ribosome feedback inhibition. On the other hand these cells do not *trans*-activate, so transcription in both cell types reaches approximately the same value. In accordance with a different extent of ribosome feedback in wild-type and *fis* cells are the results obtained upon deleting the UAS of the reporter operon. In wild-type cells expression of the operon drops to very low levels, whereas in *fis* cells the expression remains at the same level for longer periods of time (Fig. 2B).

When stable RNA synthesis is studied in exponentially growing cells at various growth rates by altering the composition of the medium, a linear relationship between the expression of stable RNA operons and the growth rate is found (11, 24). Both ribosome feedback and *trans* activation

govern this growth rate-dependent regulation, as is evident from Fig. 6, in which the expression of the *thrU* (*tufB*) operon was investigated. In accordance with previous studies of the *rrnB* operon (24), growth-rate dependent control is observed when the *thrU* (*tufB*) operon is studied after deletion of the UAS. From this finding one should not conclude, however, that ribosome feedback control is the only mechanism underlying growth rate-dependent regulation. The UAS⁺-to-UAS⁻ ratios measured at various growth rates clearly indicate that both the positive and the negative control mechanisms are operative under these conditions.

Finally the question of whether the bacterial cell benefits from harboring a *fis* gene may be asked. First, there are two counteracting regulatory systems; a positively operating *trans* activation and a negatively operating ribosome feedback may allow fine tuning of tRNA and rRNA synthesis. A prerequisite of such a balanced control is that *trans* activation, like ribosome feedback, responds to environmental signals. The results of the present study demonstrate that this condition is fulfilled. Second, FIS enables the cell to grow very fast. This may be quite an advantage when cells grow under the conditions prevailing in nature.

Until recently the *E. coli* protein FIS was known only to play a role as a host factor in the replication of certain phages. As such it did not seem to contribute to the welfare of the bacterial cell. It has become evident now that cells do benefit from this heat-stable protein and are able to switch on its synthesis when environmental conditions make this desirable.

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