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Trigger factor is induced upon cold shock and enhances viability of *Escherichia coli* at low temperatures

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ABSTRACT **Trigger factor (TF) in *Escherichia coli* is a molecular chaperone with remarkable properties: it has prolyl-isomerase activity, associates with nascent polypeptides on ribosomes, binds to GroEL, enhances GroEL's affinity for unfolded proteins, and promotes degradation of certain polypeptides. Because the latter effects appeared larger at 20°C, we studied the influence of temperature on TF expression. Unlike most chaperones (e.g., GroEL), which are heat-shock proteins (hsps), TF levels increased progressively as growth temperature decreased from 42°C to 16°C and even rose in cells stored at 4°C. Upon temperature downshift from 37°C to 10°C or exposure to chloramphenicol, TF synthesis was induced, like that of many cold-shock proteins. We therefore tested if TF expression might be important for viability at low temperatures. When stored at 4°C, *E. coli* lose viability at exponential rates. Cells with reduced TF content die faster, while cells overexpressing TF showed greater viability. Although TF overproduction protected against cold, it reduced viability at 50°C, while TF deficiency enhanced viability at this temperature. By contrast, overproduction of GroEL/ES, or hsps generally, while protective against high temperatures, reduced viability at 4°C, which may explain why expression of hsps is suppressed in the cold. Thus, TF represents an example of an *E. coli* protein which protects cells against low temperatures. Moreover, the differential induction of TF at low temperatures and hsps at high temperatures appears to provide selective protection against these opposite thermal extremes.**

Trigger factor (TF) is an abundant protein in *Escherichia coli* whose *in vivo* importance has remained unclear for a long time. It was originally isolated as a factor that bound to proOmpA protein and promoted its translocation into membrane vesicles *in vitro* (1, 2). However, subsequent studies failed to demonstrate any role of TF in protein secretion *in vivo* (3). Recently, however, TF has been shown to have a number of other remarkable properties (4). Studies from several laboratories have indicated that TF may function as a molecular chaperone that promotes the folding of nascent polypeptides (5, 6). TF is tightly associated with the 50S ribosomal particle (7), and can be cross-linked to nascent polypeptide chains (5, 6). In addition, TF was recently shown to be one of several *E. coli* peptidyl-prolyl isomerases that can catalyze the cis/trans isomerization of Xaa-Pro peptide bonds in polypeptides (8, 9). This reaction is often a rate-limiting step in the folding of certain polypeptides, such as RNaseT1, especially at low temperatures (8). In the course of studies of protein degradation, we made the unexpected discoveries that TF functions together with the major chaperones, GroEL and GroES, in the selective degradation of certain polypeptides (10) and that TF is a regulator of GroEL function (11). In fact, a fraction of the cell's TF is normally associated with GroEL, and these GroEL–TF complexes show much higher affinity for many unfolded proteins than GroEL alone (10, 11). Furthermore, increasing TF content in cells was found to enhance GroEL's ability to bind to certain unfolded proteins (10, 11), and the addition of purified TF to GroEL *in vitro* increases GroEL's binding capacity for these proteins (11). This enhancement of GroEL binding can account for its ability to stimulate the degradation of certain proteins (10) but may also be important in promoting protein folding and assembly.

Despite these seemingly important biochemical effects, increasing or decreasing TF levels was found not to affect the cell's growth rate or to have major physiological consequences at 37°C (3). In fact, the only clear *in vivo* effect was an increase in filamentation and mucoidity which was seen when TF levels were either increased or reduced (3). By contrast, the major molecular chaperones in *E. coli* (e.g., DnaK and its cofactors, GroEL and GroES) are not only essential for normal growth at 37°C, but are also heat-shock proteins that are further induced at high temperatures and by other harsh conditions that cause damage to cell proteins (12, 13). These chaperones can prevent protein aggregation, help catalyze protein refolding, and can promote the selective degradation of heat-damaged polypeptides (12–14). Unlike most molecular chaperones, TF is not a heat-shock protein and is not essential for viability at high temperatures (3). On the contrary, we had found that the effects of TF on protein degradation (10) and on GroEL's binding to proteins (11) were much greater when cells were grown at 20°C than at 37°C. For this reason, we set out to determine whether the expression and physiological importance of TF may increase at low temperatures. These studies have demonstrated a critical role for TF in maintaining cell viability at low temperatures, but also have uncovered several unexpected findings concerning cellular adaptations to high and low temperatures.

METHODS AND MATERIALS

Standard methods used throughout this study are described in the captions to specific experiments. All strains and their origins have been described previously (11).

RESULTS

Cellular Content of TF Rises with Decrease in Growth Temperature. To test whether TF content increases at low temperatures, the wild-type C600 strain was grown to mid-log phase in Luria–Bertani (LB) medium at temperatures ranging from 16°C to 42°C. When the cells reached the same optical density, cell proteins were analyzed by SDS/PAGE, and the amounts of TF were measured by Western blot analysis with an anti-TF antibody and ^{125}I-protein A. For comparison, we also measured the levels of the major heat-shock protein,

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Abbreviations: TF, trigger factor; hsp, heat-shock protein; LB, Luria–Bertani; IPTG, isopropyl β-D-thiogalactoside.

GroEL, by a similar approach using an anti-GroEL antibody. At 42°C, when GroEL content reached its maximum, TF content was lowest. By contrast, at 16°C, TF content was 2- to 3-fold higher than in the culture growing at 37°C, even though GroEL content fell significantly in cultures grown below 27°C (Fig. 1*A*). Furthermore, when cells growing logarithmically at 37°C were transferred to 4°C for 48 hr, TF content also increased about 2-fold (Fig. 1*B*). Thus, although GroEL and TF can function together (10, 11), their expression is regulated in opposite fashions, and TF appears to be induced by low temperatures.

TF Synthesis Is Induced upon Temperature Downshift or Exposure to Chloramphenicol. Because the cellular content of TF increased as growth temperature fell, we studied whether this increase was due to enhanced synthesis. After the shift from 37°C to 10°C, growth of the culture ceased for about 4 hr, after which the bacteria reinitiated exponential growth, but at a lower rate, in accord with prior observations (15, 16). After temperature down-shift, during the lag-period, *E. coli* synthesize primarily a unique set of cold-shock proteins (16, 17). To test whether TF is one of these proteins, rates of incorporation of [^{35}S]methionine into TF were measured before and after the shift to 10°C. Equivalent amounts of labeled cell proteins were analyzed by immunoprecipitation with a specific anti-TF antibody followed by SDS/PAGE and autoradiography (Fig. 2*A*). During the first 2 hr at 10°C, the differential rate of TF synthesis decreased (i.e., the rate as a fraction of total protein synthesis), but then it increased markedly, and during the third and fourth hours, TF synthesis was at least 2-fold higher than at 37°C. With the subsequent resumption of growth at 10°C, the differential rate of TF synthesis remained slightly higher than at 37°C (data not shown). A similar sequence of changes in TF synthesis was found upon shift of the cells from 37°C to 16°C (data not shown). This pattern of changes in TF synthesis resembles exactly the pattern characteristic of cold-shock proteins in *E. coli* (16).

Previous studies have shown that a number of cold-shock proteins can be induced at 37°C by a specific group of antibiotics that reduce the rate of translation, including chloramphenicol, tetracycline, erythromycin, spiramycin, and fusidic acid (18). To test whether TF synthesis is also stimulated under these conditions, wild- type *E. coli* were grown at 37°C to mid-log phase and chloramphenicol added at a final concentration of 20 μg/ml, which reduced, but did not prevent, cell growth. As shown in Fig. 2*B*, the differential rate of TF synthesis increased by approximately 2-fold by 30 min after chloramphenicol addition. By contrast, the differential rate of synthesis of GroEL was reduced in the presence of chloramphenicol (data not shown), in accord with prior findings (18, 19). Thus, synthesis of TF is regulated in a similar way as that of other cold-shock proteins and in an opposite fashion to the synthesis of hsps.

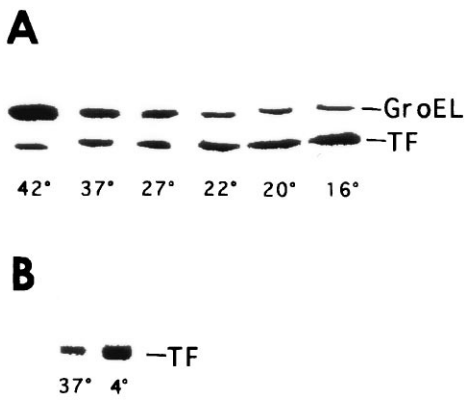

FIG. 1. (*A*) Cellular content of TF, unlike that of GroEL, increases as growth temperature decreases. C600 cells were grown in LB medium at different temperatures to the same optical density (A_{600} = 0.5). Equal aliquots were taken, and cells collected by centrifugation. Cell proteins were resolved by SDS/PAGE, and the relative amounts of TF and GroEL measured by Western blot analysis with an anti-TF or anti-GroEL antibody and ^{125}I-protein A. (*B*) TF content decreases at 4°C. C600 cells were grown in LB medium to A_{600} = 0.5 at 37°C and transferred to 4°C. Equal aliquots were taken from the cell culture before and 48 hr after the temperature shift, and TF content was measured as described above.

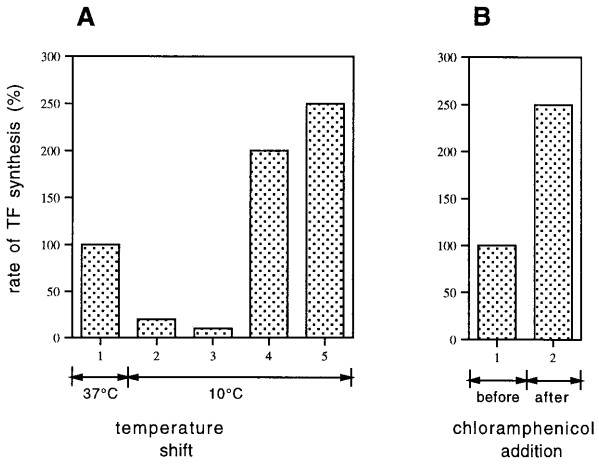

FIG. 2. Differential rate of TF synthesis increases at low temperatures or in the presence of cloramphenicol. (*A*) C600 cells were grown in Davis minimal medium supplemented with amino acids and glucose until mid-log phase at 37°C and transferred to 10°C. To label cell proteins, 1 ml aliquots were taken at different times and incubated with [^{35}S]methionine (10 μCi/ml; 1 Ci = 37 GBq) for 5 min at 37°C (lane 1) and for 60 min at the following times after the shift to 10°C: 0–60 (lane 2), 60–120 (lane 3), 120–180 (lane 4), and 180–240 min (lane 5). Cells were collected by centrifugation, and proteins solubilized by boiling in a buffer containing 0.3% SDS (10). After a 50-fold dilution with the immunoprecipitation buffer (10) to reduce the SDS concentration, equivalent amounts of radioactive cell proteins were used for immunoprecipitation with a specific anti-TF antibody and protein A-trisacryl (10). The amount of radioactive TF in the immunoprecipitates was then determined by SDS/PAGE followed by autoradiography. (*B*) C600 cells were grown in Davis minimal medium supplemented with amino acids and glucose until mid-log phase at 37°C, and chloramphenicol was added to a final concentration of 20 μg/ml. Cell proteins were labeled for 5 min with [^{35}S]methionine (10 μCi/ml) just before and 30 min after chloramphenicol addition. The amount of radiolabeled TF was determined as described in *A*.

TF Protects Cells Against Low Temperatures. Prior studies have failed to demonstrate a requirement, or even a clear influence, of TF on cell growth at 37°C (3). Because TF content rises with decreasing growth temperature, and because the effects of TF on GroEL function were more pronounced at 20°C than at 37°C (10, 11), we tested whether this protein might be especially important for cell viability at low temperatures. We used strains (kindly provided by W. Wickner, Dartmouth Medical School) that express TF at either very high or very low levels (3). The TF-overexpressing strain had the *tig* gene on a multicopy plasmid (pTIG2) under the regulation of the arabinose promoter. Therefore, in the presence of arabinose, the content of TF increased up to 10-fold (3). Cells expressing low levels of TF had the *tig* gene integrated into the chromosome under the control of the *ara* promoter. When the cells were grown in medium containing glucose instead of arabinose, TF synthesis was repressed, such that under the present experimental conditions its level was reduced by over 90% (3). When these strains were grown in the presence of arabinose or glucose at 20°C, 30°C, 37°C, or 42°C, they grew at similar rates as wild-type cells in the same media at these temperatures.

To learn whether TF content affects viability at very low temperatures that prevent normal growth, wild-type, TF-overproducing, and TF-deficient strains were grown until mid-log phase at 37°C, and then were plated on Petri dishes and incubated at 4°C. At different times, the number of colonies surviving at 4°C were measured by transferring the plates to 37°C. As shown in Fig. 3*A*, all these cultures lost viability exponentially, but at quite different rates. The wild-type cells died with a half-life of 4–5 days whether they were grown in arabinose- or glucose-containing medium (Fig. 3), and after a week at 4°C, about 15% of the cells remained alive. However, only 1% of the TF-deficient cells survived after a week at 4°C ($t_{1/2}$ = 1–2 days). Thus, decreased TF content markedly reduces cell viability in the cold. By contrast, 40% of the TF-overexpressing cells were still viable at this time ($t_{1/2}$ = 6–7 days). In different experiments, the extent of increase in TF content ranged from 3- to 10-fold. Nevertheless, a similar degree of protection against the cold was seen in these different experiments. Thus, the 2- to 3-fold induction seen typically upon cold shock of wild-type cells is likely to enhance viability at low temperatures.

Heat-Shock Proteins Are Harmful to Cells at 4°C. It is well established that induction of many molecular chaperones, as part of the heat-shock response, helps protect cells against high temperatures and a number of other harsh conditions (12–14). Similar experiments were therefore carried out to test whether cells carrying high levels of hsps generally, or just high levels of GroEL and GroES, are also protected against loss of viability at 4°C. To increase the production of the hsps generally, we used cells that carry either the heat-shock-specific σ-factor, $^{32}\sigma$, or the *groEL/ES* operon, on a plasmid under the control of the *lac* promoter. Interestingly, when such cells were grown at 37°C in the presence of IPTG and then shifted to 4°C, both cultures actually lost viability much faster than wild-type cells (Fig. 3*B*). The cells overexpressing all hsps lost viability with a half-life of about 1 day, and those overexpressing GroEL/ES with a half-life of 3 days, compared with 5 days for wild type. Similar effects on viability were obtained in different experiments where the degree of increase in GroEL and DnaK content varied from 3- to 10-fold (due to different length of induction by IPTG). These findings suggest that TF and hsp provide protection against different thermal extremes: the induction of TF protects at low temperatures, while hsps are induced as temperature rises and protect cells against high temperatures (12–14).

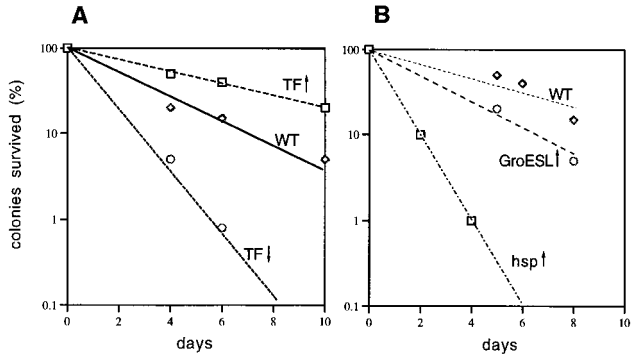

FIG. 3. Effects of increasing or decreasing expression of TF (*A*) and hsp generally or GroEL/ES (*B*) on cell viability at 4°C. (*A*) TF-overproducing strain was grown until mid-log phase at 37°C in LB medium supplemented with arabinose which increased TF content from 3- to 10-fold (depending on the experiment). The TF-deficient cells were grown in the presence of glucose which reduced TF content 10-fold, as described (3, 10). In each case, wild-type controls were grown under similar conditions. The cultures were diluted with LB medium, and equal amounts of cells from each were plated on the Petri dishes containing arabinose or glucose, respectively. The plates were stored at 4°C, and after different times, the number of colonies that survived were measured by transferring the plates to 37°C. Very similar results were obtained when the liquid cultures were stored at 4°C, at different times plated on the Petri dishes, and the colonies that grew at 37°C were counted. (*B*) To increase cellular content of heat-shock proteins, wild-type cells carrying pUHE211–1 plasmid containing hsp-specific subunit of RNA polymerase, σ32, under the control of *lac* promoter (kindly provided by B. Bukau, University of Heidelberg), were grown to mid-log phase in LB at 37°C. Then the culture was divided into two parts. Isopropyl β-D-thiogalactoside (IPTG; 1 mM) was added to one of them, and both cultures were incubated for 0.25–1 hr at 37°C which resulted in a 3- to 10-fold increase in hsp content (depending on the experiment). To study the effects of overproducing GroEL/ES, similar experiments were carried out with cells carrying the *groES/EL* operon on a plasmid (pDK84, kindly provided by M. Snavely, Amgen Biologicals) under the control of the *lac* promoter. The effect of hsp overproduction on cold survival was studied as described in *A*.

TF Reduces Viability at Very High Temperatures. Because of TF's ability to protect against the cold, we tested whether increased TF content might also enhance cell viability at elevated temperatures or might even reduce viability (since cell content of TF was found to decrease as temperature rose). The TF-overexpressing, TF-underexpressing, and wild-type strains were grown to mid-log phase and then shifted to 50°C, where *E. coli* die rapidly. Aliquots were taken from each culture at 5-min intervals and plated on Petri dishes at 37°C to determine the number of cells remaining viable. At 50°C, wild-type cells lost viability with a half-life of 20 min (Fig. 4). In contrast to the protection observed at 4°C, at 50°C, the cells containing high amounts of TF died much faster (half-life of 7 min), while those with reduced TF levels survived longer (half-life of 35 min) than did the wild type (Fig. 4). Thus, the induction of TF at high temperatures has the opposite effects as heat-shock proteins. These findings suggest that it would be advantageous for the cell to reduce its TF content at elevated temperatures, and, in fact, TF content at 42°C is clearly lower than at 37°C (Fig. 1).

DISCUSSION

These studies have demonstrated that TF, unlike the major molecular chaperones in *E. coli*, is induced at low temperatures and is essential for cell viability in the cold. Interestingly, overproduction of TF and induction of hsps generally (or just GroEL or GroES alone) were found to have opposite effects on cell survival at very low and at very high temperatures, both of which prevent growth and cause cell death (Fig. 3). Thus, TF and the hsps must protect cell proteins against distinct types of damage. TF, and presumably other cold-shock proteins, can help protect cells against the effects of low temperature. In

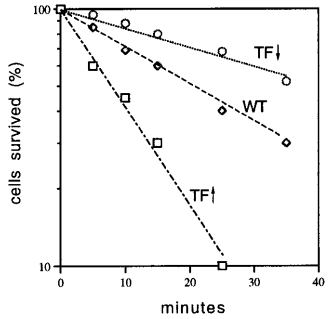

FIG. 4. Effects of increasing or decreasing expression of TF on cell viability at 50°C. TF-overproducing, TF-underproducing, and wild-type strains were grown until mid-log phase at 37°C in LB medium supplemented with arabinose to induce TF overexpression or glucose to inhibit TF expression, as in Fig. 3*A*. The cultures were then shifted to a water-bath at 50°C. Aliquots were taken from each culture before and at different times after shift to 50°C. Cells were diluted and plated on Petri dishes at 37°C to determine the number of surviving bacteria.

fact, an enhancement of viability was seen when TF content was increased only about 3-fold, which is the degree of induction that was seen upon shift of wild-type cells to 10°C or 16°C for several hours. On the other hand, hsps help protect cell proteins from irreversible denaturation or covalent damage induced by high temperatures, ethanol, oxygen radicals, and heavy metals (12–14). These effects on viability at high temperatures can explain why expression of GroEL and GroES is greatest above 40°C and is suppressed below 27°C, and why their synthesis is regulated in an opposite fashion to that of TF (Fig. 1*A*). However, the biochemical reason(s) why the hsps help protect cells against high temperatures but reduce viability at low temperatures are quite unclear. It is also not known why TF induction reduces viability at 50°C. Whatever the explanation is, this protection against high temperatures by decreased TF content can explain why cellular levels of TF fall above 37°C. Apparently, the biochemical requirements for thermotolerance and cold-tolerance are distinct and mutually exclusive.

One likely signal for induction of cold-shock proteins upon temperature downshift is the reduced rate of translation, since a similar induction (and a repression of hsps) occurs with exposure to certain antibiotics that slow the translation process (18). Approximately 14 polypeptide spots have been identified as cold-shock proteins in two-dimensional electrophoretic analysis of *E. coli* (17). Following a shift to low temperatures, the synthesis of one of these proteins, CspA, is induced rapidly and dramatically (up to 200-fold) (16) and promotes transcription of other cold-shock proteins (19). However, upon temperature downshift, induction of other cold-shock proteins is more modest (2- to 10-fold) and occurs after a lag of 2–3 hr (16, 17, 19). TF expression appears typical of this latter group of proteins. It has been proposed that these proteins are important in enabling the cells to grow at low temperatures. However, TF is the only protein thus far shown actually to enhance viability in the cold.

Among the cold-shock proteins are several components of the cell's transcriptional and translational machinery (20). Because of TF's association with ribosomes (7) and growing polypeptides (5, 6), TF induction in the cold may represent an adaptation that allows protein synthesis and folding to continue at low temperatures, where the solubility, aggregation, and folding properties of proteins are quite different than at 37°C or 42°C. Alternatively, TF may be important in maintaining preexistent cell proteins in a functional form—i.e., by promoting the refolding of cold-damaged proteins. Such a "maintenance" or "repair" function may be particularly important at low temperatures where synthesis of new proteins proceeds very slowly. Another molecular chaperone in *E. coli*, Hsc66, was recently shown also to be induced by low, but not high, temperatures (21). This newly discovered protein (22, 23) is an Hsp70 homolog whose expression is regulated in an opposite fashion to that of the major hsp70 homolog, DnaK, a classic heat-shock protein. In yeast, there are also hsp70 family members that are induced at high temperatures (SSA) and ones induced at low temperatures (SSB) (24). These findings further suggest that at low temperatures, protein folding (or refolding) requires distinct enzymatic machinery than at high temperatures.

The exact reason(s) why cells die at 4°C is unclear and, to our knowledge, has not been studied. Interestingly, this process follows first-order kinetics and thus represents a random decay process. It is also not clear which particular function(s) of TF are important for enhancing cell survival in the cold. The present observations suggest that this effect is not through TF's ability to enhance GroEL function (10, 11); otherwise, cell killing at 4°C would have been enhanced by TF overproduction as occurred with GroEL overproduction. An attractive alternative possibility is that the critical property of TF at low temperature is its prolyl isomerase activity (8). The temperature-dependence of proline isomerization is very large, compared with other steps in protein folding, and it can be a rate-limiting step for protein folding at low temperatures, when spontaneous isomerization of proline is quite slow (8). In addition to the peptidyl-prolyl isomerases domain (8, 25), TF contains a separate protein-binding domain that behaves as a chaperone in facilitating protein binding and folding (26). Future studies with TF mutants may clarify the importance of the peptidyl-prolyl isomerase activity or other domains in protecting cells against low temperatures.

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