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The *Escherichia coli mazEF* Suicide Module Mediates Thymineless Death

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In 1954, Cohen and Barner discovered that a thymine auxotrophic (*thyA*) mutant of *Escherichia coli* undergoes cell death in response to thymine starvation. This phenomenon, called thymineless death (TLD), has also been found in many other organisms, including prokaryotes and eukaryotes. Though TLD has been studied intensively, its molecular mechanism has not yet been explained. Previously we reported on the *E. coli mazEF* system, a regulatable chromosomal suicide module that can be triggered by various stress conditions. MazF is a stable toxin, and MazE is an unstable antitoxin. Here, we show that cell death that is mediated by the *mazEF* module can also be activated by thymine starvation. We found that TLD depends on *E. coli mazEF* and that under thymine starvation, the activity of the *mazEF* promoter P_2 is significantly reduced. Our results, which describe thymine starvation as a trigger for a built-in death program, have implications for programmed cell death in both prokaryotes and eukaryotes.

As early as 1954, Cohen and Barner discovered that a thymine auxotrophic (*thyA*) mutant of *Escherichia coli* undergoes cell death in response to thymine starvation (10). This phenomenon, called thymineless death (TLD) (7), has also been found in many other organisms, including prokaryotes and eukaryotes (for a recent review, see reference 2). TLD is a unique effect, since starvation of bacteria for the other bases (adenine, guanine, or cytosine) or other nutrients, like amino acids or vitamins, causes growth to stop (i.e., is bacteriostatic) but does not cause cell death (i.e., is not bactericidal) (6). However, despite intensive research on TLD, its molecular mechanism has not yet been explained. Though many molecular events have been implicated in TLD, no compelling direct relationship has been found among them, and thus, no definite hypothesis has been formulated (2).

Most of the studies of TLD have been made in *E. coli*, where the first step in dTTP synthesis is the conversion of dUMP to dTMP. This conversion is catalyzed by thymidylate synthetase, which is encoded by the *thyA* gene (5), and involves the transfer of a C-1 unit from N^5,N^{10}-methylene tetrahydrofolate to the 5 position of dUMP (2); tetrahydrofolate is consumed in this reaction (15). Thus, thymine starvation in *E. coli* can be induced in several ways that inhibit the synthesis of thymidylate either directly or indirectly by interfering with folate metabolism (2).

We asked whether thymine starvation is a trigger for a built-in death program. In *E. coli*, programmed cell death (PCD) is mediated through special genetic elements called addiction modules (for reviews, see references 11, 13, 16, and 31). These modules consist of two genes, where the second gene encodes a stable toxin and the first gene encodes a labile antitoxin. Addiction modules were first discovered in a number of extrachromosomal elements, where they were found to be responsible for the postsegregational killing effect, that is, the death of cells from which these extrachromosomal elements have been lost. Thus, the cells are "addicted" to the short-lived antitoxin product, since its de novo synthesis is essential for cell survival (11, 13, 16, 31).

Pairs of genes homologous to some of these extrachromosomal addiction modules have been found on the *E. coli* chromosome (3, 16, 17, 21–23). The *E. coli mazEF* system, the first known regulatable prokaryotic chromosomal addiction module, has been reported (3). The *mazEF* module consists of two adjacent genes, *mazE* and *mazF*, located in the *rel* operon downstream from the *relA* gene (23). In the study (3), it was found that *mazEF* has the properties required for an addiction module: (i) MazF is toxic, and MazE is antitoxic; (ii) MazF is long-lived, while MazE is a labile protein degraded in vivo by the ATP-dependent ClpPA serine protease; (iii) MazE and MazF interact; (iv) MazE and MazF are coexpressed; and (v) The two *mazEF* promoters (P_2 and P_3) are strongly negatively autoregulated by the combined action of MazE and MazF (20). Another significant characteristic of the *mazEF* system is that its transcription from the P_2 promoter is inhibited by high concentrations of guanosine 3′,5′-bispyrophosphate (ppGpp) (3), which is synthesized by the RelA protein under conditions of extreme amino acid starvation (9). Based on these properties of *mazEF*, and on the requirement for the continuous expression of MazE to prevent cell death, a model for PCD under conditions of nutritional stress (3) or under conditions in which the synthesis of RNA and/or protein are generally inhibited (19, 27) was presented. The model was supported by the results of previous experiments showing that *mazEF*-mediated cell death can be triggered by (i) the artificial overproduction of ppGpp (3, 14), (ii) several antibiotics (rifampin, chloramphenicol, and spectinomycin) that are general inhibitors of transcription and/or translation (12, 27), or (iii) the Doc protein, the toxic product of the addiction module *phd-doc* of

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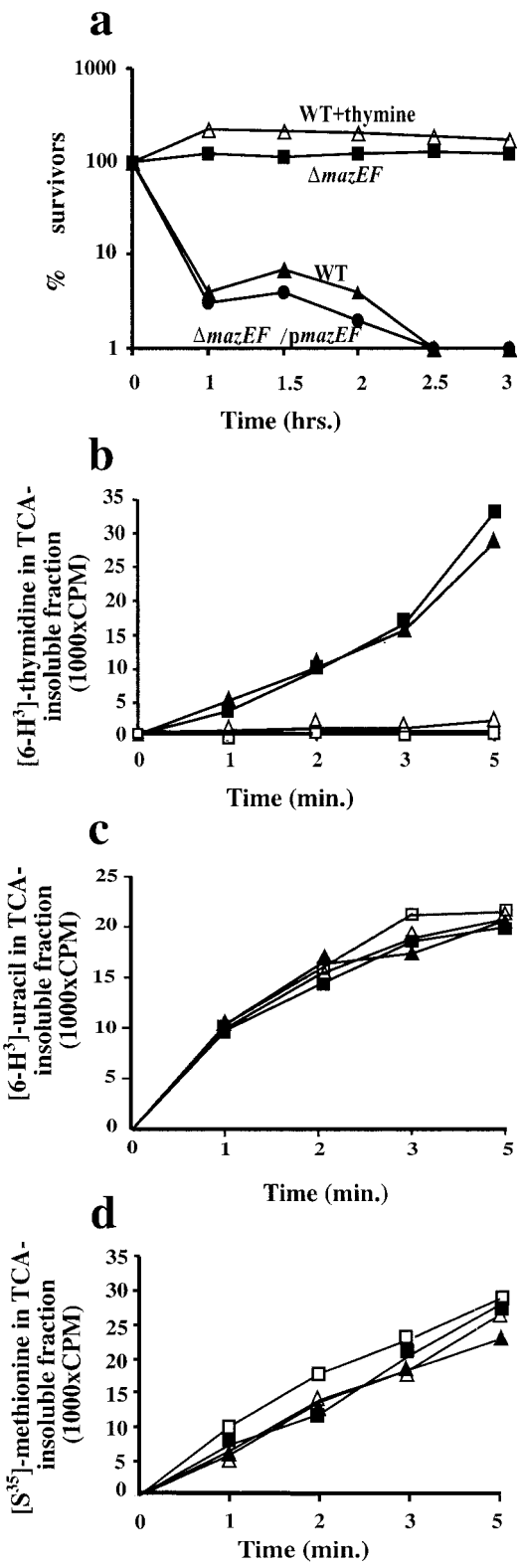

FIG. 1. (a) Thymine starvation by trimethoprim in *E. coli* induces *mazEF*-mediated cell death. Cells were grown at 37°C in M9 liquid minimal medium plus 0.1 μg of trimethoprim/ml as described in Materials and Methods. Viability (% survivors) is plotted against the time of exposure to trimethoprim of *E. coli* MC4100*relA*$^+$ (WT), MC4100Δ*mazEFrelA*$^+$ (Δ*mazEF*), and MC4100Δ*mazEFrelA*$^+$/p*mazEF* (Δ*mazEF*/p*mazEF*). MC4100*relA*$^+$ was also grown in the presence of 0.1 μg of trimethoprim/ml plus 50 μg of thymine/ml (WT+thymine). We calculated cell survival by comparing the colony-forming ability of trimethoprim-treated cells to that of untreated cells at zero time. The numbers represent the results of one out of five similar experiments. (b to d) Effect of thymine starvation on the synthesis of DNA (b), RNA (c), and protein (d) in MC4100*relA*$^+$ and its Δ*mazEF* derivative. Cells were grown at 37°C in M9 liquid medium as described in Materials and Methods. After overnight growth, the cultures were diluted to 10^7 cells/ml and allowed to grow for 1 h in M9 medium plus inosine (50 μg/ml). Then, trimethroprim (2 μg/ml) was added; the cells were immediately labeled with either [6-^{3}H]thymidine (b), [5,6-^{3}H]uracil (c), or [^{35}S]methionine (d); and their incorporation into the acid-insoluble fraction was determined as described in Materials and Methods. As controls, we used MC4100*relA*$^+$ (▲) and its Δ*mazEF* derivative (■) without the addition of trimethoprim. △, trimethoprim-treated MC4100*relA*$^+$; □, Δ*mazEF* derivative.

the plasmid prophage P1, which is a general inhibitor of translation (19).

Here, we asked whether *mazEF*-mediated cell death can be triggered in *E. coli* under conditions of thymine starvation. We generated the condition of thymine starvation in three ways: (i) we caused the inhibition of folate metabolism by adding trimethoprim to inhibit dihydrofolate reductase (4), (ii) we caused the inhibition of folate metabolism by adding sulfonamides to block folate synthesis (28), and (iii) we grew a thymine auxotrophic (*thyA*) mutant in a medium lacking thymine (1, 10). We found that in *E. coli* thymine starvation does trigger *mazEF*-mediated cell death. Furthermore, under thymine starvation, the activity of the *mazEF* promoter P_2 is significantly reduced. Thus, the results of our work showing that thymine starvation is a trigger for a built-in death program provide a new insight into an old enigma.

MATERIALS AND METHODS

Materials and media. We obtained [6-^{3}H]thymidine (23.0 Ci/mmol) and [5,6-^{3}H]uracil (45 Ci/mmol) from Amersham Pharmacia Biotech (Little Chalfont, United Kingdom) and [^{35}S]methionine (1,175 Ci/mmol) from NEN (Boston, Mass.). Trimethoprim, sulfamethoxazole (SMZ) and inosine were obtained from Sigma (St. Louis, Mo.). We obtained avian myeloblastosis virus reverse transcriptase from Promega (Madison, Wis.), we obtained polynucleotide kinase from U.S. Biochemical Corp. (Cleveland, Ohio), and we obtained [γ-^{32}P]ATP (3,000 Ci/mmol) from Amersham Pharmacia Biotech. The bacteria were grown either in M9 liquid medium with 1% glucose and a mixture of amino acids (20 μg/ml each) or in Luria-Bertani (LB) liquid medium and/or agar plates (24).

Bacterial strains and plasmids. We used the following *E. coli* strains: MC4100*relA*$^+$ [*araD139* Δ(*argF-lac*)*205 flb5301 pstF25 rpsL150 deoC1*) and its derivative MC4100Δ*mazEFrelA*$^+$ (*mazEF*::*kan*) (14). In addition, we used KL742 (λ$^-$ *thyA748*::Tn*10 rph-1 deo-77*) (from the *E. coli* Genetic Stock Center) (K. B. Low, unpublished strains). Its Δ*mazEF* derivative KL742*mazEF*::*kan* and its Δ*clpP* derivative KL742*clpP*::*cat* were constructed by P1 transduction from strains MC4100*mazEF*::*kan* and MC4100*clpP*::*cat* (3), respectively.

Plasmid pKK223*mazEF* (p*mazEF*) is a pKK223 derivative carrying *mazEF* (19). Plasmids pSK10Δ6-p_{ef} and pIB13 are derivatives of plasmid pSK10Δ6, which bears the *lac′Z* gene lacking its first eight codons. pSK10Δ6-p_{ef} carries the end of the *relA* gene, the *mazEF* promoter, and the first 17 codons of *mazE* fused to *lac′Z* (20). pIB13 carries the *trpR* promoter and the first 78 codons of *trpR* fused to *lac′Z* (8).

Thymine starvation induced by the addition of trimethoprim or SMZ. Bacteria were grown in M9 minimal medium (with glucose and a mixture of amino acids) with shaking (160 rpm) at 37°C. After overnight growth, the cultures were (i) diluted to 10^6 cells/ml in M9 medium supplemented with trimethoprim (0.1 μg/ml) and inosine (50 μg/ml) or (ii) diluted in M9 medium supplemented with SMZ (10 μg/ml) and inosine (50 μg/ml). Inosine was added as a purine source (28). While these diluted cultures were growing at 37°C, samples were withdrawn at 30-min intervals, washed once with saline, and plated on LB plates that were incubated at 37°C for 18 h for viable-cell counts.

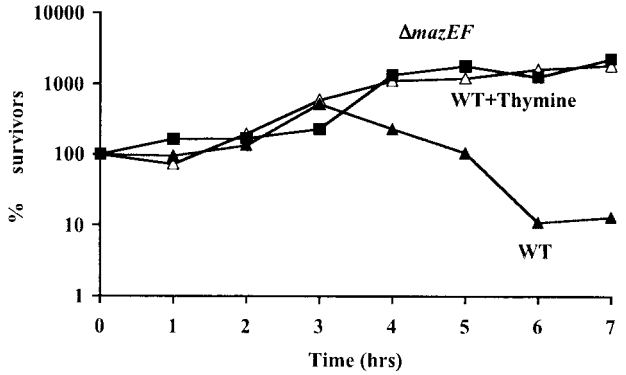

FIG. 2. Thymine starvation by SMZ in *E. coli* induces *mazEF*-mediated cell death. Viability (% survivors) is plotted against time of exposure to SMZ (10 μg/ml) of *E. coli* MC4100*relA*$^+$ (WT) and its Δ*mazEF* derivative (Δ*mazEF*) in M9 liquid medium at 37°C. MC4100*relA*$^+$ was also grown in the presence of 10 μg of SMZ/ml plus 50 μg of thymine/ml (WT+Thymine). Cell survival was determined as described in the legend to Fig. 1a.

Thymine starvation of a thymine auxotrophic (*thyA*) mutant. The *E. coli thyA* mutant strain KL742 was grown in M9 minimal medium supplemented with thymine (50 μg/ml) plus glucose and a mixture of amino acids with shaking (160 rpm) at 37°C. After overnight growth, the cultures were diluted 20-fold in the same medium and allowed to continue growing with shaking at 37°C. When the culture reached the exponential phase (10^8 cells/ml), 1.0-ml samples were withdrawn, centrifuged, washed two times with saline at 4°C, and resuspended in 2.0 ml of fresh M9 medium without thymine. These prepared samples were incubated at 37°C with aeration (shaking at 160 rpm). At 30-min intervals, 0.1-ml samples were withdrawn, diluted appropriately, and plated on LB nutrient agar plates supplemented with thymine (50 μg/ml).

Assays for the effects of thymine starvation on the synthesis of DNA, RNA, and protein. Synthesis of DNA, RNA, and protein was determined by measuring the incorporation of [6-^{3}H]thymidine (0.2 μCi/ml), [5,6-^{3}H]uracil (0.1 μCi/ml), or [^{35}S]methionine (24 μCi/ml), respectively, into a trichloroacetic acid (TCA)-insoluble fraction. We added this radioactive material to the cultures at the beginning of the period of thymine starvation, induced by growing the cells in M9 medium in the presence of trimethoprim and inosine as described above. At various times during thymine starvation, the reactions were stopped by adding TCA to 1-ml samples to a final concentration of 10%, and the mixtures were incubated on ice for 30 to 60 min. The precipitates were filtered on Millipore (Bedford, Mass.) filters and washed with TCA (5%). The TCA solutions contained 100 μg of nonradioactive thymidine or nonradioactive uracil/ml. The filters were allowed to dry, and the TCA-insoluble counts were determined by using a scintillation counter.

Assay for the effect of thymine starvation on the activity of the *mazEF* promoter P_2 and the *trpR* promoter. *E. coli* strain MC4100*relA*$^+$ harboring either plasmid pSK10Δ6-p_{ef} or plasmid pIB13 was grown in LB medium to 10^8 cells/ml, and trimethoprim (5 μg/ml) was added. At various times, RNA was extracted, and primer extension was carried out with avian myeloblastosis virus reverse transcriptase as described previously (20). The oligonucleotide primer used for the pSK10Δ6-p_{ef} construct was from positions −40 to −23 of the *lacZ* gene toward the transcription start site of *mazEF* (20). The primer used for pIB13 was from positions 82 to 59 of the *trpR* gene toward the promoter region of *trpR* (18). The primers were end labeled by polynucleotide kinase with [γ-^{32}P]ATP. The reaction products were resolved on a 6% sequencing gel, and DNA-sequencing reactions were performed with the same primers and were run in parallel with the primer extension reaction as described previously (20). To quantify the RNA levels, the gels were analyzed and the bands were quantified using the Fujix BAS100 phosphorimager.

RESULTS

Thymine starvation by trimethoprim induces cell death through the *mazEF* system. First, we studied the effect on *mazEF*-mediated cell death of thymine starvation induced by the addition of trimethoprim. Specifically, we compared the viability of the wild-type *E. coli* strain MC4100*relA*$^+$ to that of its Δ*mazEF* derivative after exposure to trimethoprim. Growing the wild-type cells in the presence of trimethoprim caused 96% of the cells to die by 1.0 h of exposure and caused 99% of the cells to die by 2.5 h of exposure (Fig. 1a). Since the addition of thymine completely reversed this phenomenon, these data represent a TLD curve. In contrast to the wild-type strain, the Δ*mazEF* derivative did not die in the presence of trimethoprim, and close to 100% of these cells were viable even after 3.0 h (Fig. 1a). We also tested the effect of trimethoprim in Δ*mazEF* cells in which the *mazEF* gene module was present, on plasmid pKK223*mazEF* rather than on the chromosome. Here again, we observed TLD: 97% of the cells died after 1.0 h. Thus, we found that TLD induced by trimethoprim requires the *mazEF* system.

In addition, we showed that the dependence of TLD on the function of the *mazEF* system has no connection with changes in the synthesis of macromolecules, since we found that DNA synthesis was inhibited in both the wild type and the Δ*mazEF* derivative mutant (Fig. 1b), while RNA and protein synthesis were unaffected (Fig. 1c and d).

Thymine starvation by a sulfonamide induces cell death through the *mazEF* system. We then studied the effect of the induction of thymine starvation by the sulfonamide SMZ on *mazEF*-mediated cell death. We compared the viability of the wild-type *E. coli* MC4100*relA*$^+$ to the viability of its Δ*mazEF* derivative after exposing each of the strains to SMZ (Fig. 2). Death of the wild-type strain occurred later in the presence of SMZ (Fig. 2) than in the presence of trimethoprim (Fig. 1a). In particular, 90% of the wild-type strain died after 6 h of exposure to SMZ (Fig. 2). In contrast, under identical conditions, we observed no killing of the Δ*mazEF* derivative at all (Fig. 2); in fact, this Δ*mazEF* strain continued growing at the same rate as the wild-type strain when thymine was added to reverse the effect of SMZ (Fig. 2). Thus, as when we induced thymine starvation by the addition of trimethoprim (Fig. 1a), TLD was also clearly *mazEF* dependent in the presence of SMZ (Fig. 2).

Thymine starvation of a *thyA* mutant induces cell death through the *mazEF* system. Finally, we studied the involvement of the *mazEF* module in TLD induced by the lack of thymine in a thymine auxotrophic (*thyA*) mutant. For this pur

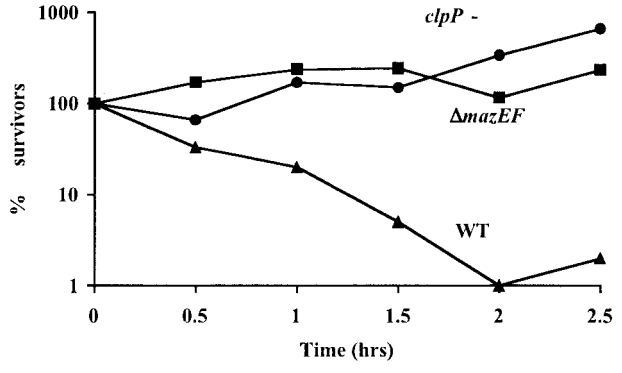

FIG. 3. Thymine starvation in an *E. coli thyA* mutant induces *mazEF*-mediated cell death. Viability (% survivors) is plotted against times of growth of *E. coli* strains KL742*thyA748*::Tn*10* (WT), KL742*thyA748*::Tn*10*Δ*mazEF* (Δ*mazEF*), and KL742*thyA748*::Tn*10* Δ*clpP* (*clpP* −) at 37°C in M9 liquid medium lacking thymine. Cell survival was determined as described in the legend to Fig. 1a.

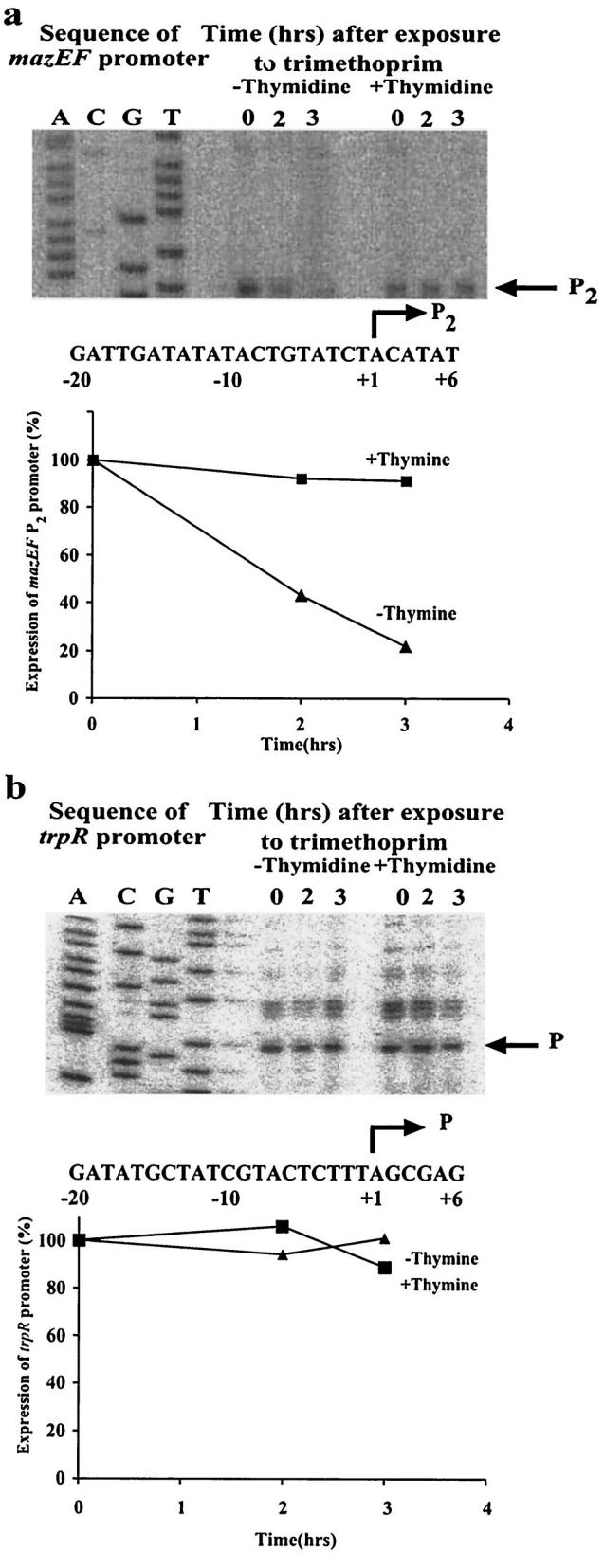

FIG. 4. (a) Thymine starvation by trimethoprim in *E. coli* MC4100*relA*$^+$ reduces transcription from the *mazEF* promoter P_2. *E. coli* strain MC4100*relA*$^+$ harboring plasmid pSK10Δ6-p_{ef} was treated with trimethoprim; RNA was extracted at the indicated times, primer extension was carried out, and the reaction products were resolved on a 6% sequencing gel as described in Materials and Methods. The arrow indicates the position of the P_2 promoter. The lanes marked A, C, G and T indicate the sequence of this promoter in plasmid pSK10Δ6-p_{ef}. The *mazEF* P_2 promoter region (nucleotides −20 to +6) (20) is illustrated. (b) Thymine starvation by trimethoprim in *E. coli* MC4100*relA*$^+$ does not affect transcription of the *E. coli trpR* promoter. *E. coli* MC4100*relA*$^+$ harboring plasmid pIB13 was treated with trimethoprim, and RNA was extracted at the indicated times. Other experimental conditions were as described for panel a and in Materials and Methods. The arrow indicates the position of the *trpR* promoter (P). The lanes marked A, C, G, and T indicate the sequence of this promoter in plasmid pIB13. The *trpR* promoter region (nucleotides −20 to +6) (18) is illustrated.

pose, we used *E. coli* strain KL742, which carries the mutation *thyA748*::Tn*10*. Here, we observed 95% cell death after 1.5 h and 99% cell death after 2.0 h of growth in the absence of thymine. However, we observed almost no killing during this period in either Δ*mazEF* or Δ*clpP* derivatives of *E. coli* KL742 *thyA* (Fig. 3). Thus, TLD induced by thymine starvation in a thymine auxotrophic mutant is both *mazEF* and *clpP* dependent.

Thymine starvation in *E. coli* reduces transcription from the *mazEF* promoter P_2. We asked whether thymine starvation affects transcription from the *mazEF* promoter. To test the *mazEF* promoter activity, we used plasmid pSK10Δ6-p_{ef}, which was previously constructed for this purpose (20). It carries a gene fusion in which the beginning of *mazE* is fused to the eighth codon of *lacZ* (see Materials and Methods). We transformed this plasmid into *E. coli* MC4100 *relA*$^+$. The cells harboring the plasmid were starved for thymine with trimethoprim, and during starvation, the activity of the *mazEF* promoter was determined by primer extension. As shown, the level of transcription from P_2 was reduced to ~25% after 2 h of thymine starvation compared to unstarved cells (Fig. 4a). We also found that similar conditions of thymine starvation by trimethoprim do not affect the activity of the *E. coli trpR* promoter (Fig. 4b).

DISCUSSION

In summary, we have shown that TLD induced in *E. coli* K-12 strains is mediated through the *mazEF clpP*-dependent system (3, 12–14, 19, 20, 27). We used three different procedures to induce thymine starvation: treatment with trimethoprim or with a sulfonamide antibiotic or thymine starvation of a *thyA* auxotroph. Moreover, we found that thymine starvation by trimethoprim drastically reduces transcription from the *mazEF* promoter P_2 (Fig. 4a), which was previously described as mainly responsible for transcription and regulation of *E. coli mazEF* (3, 20). *mazEF* has an additional promoter, P_3, located 13 nucleotides downstream from P_2, that has only 1/10 of the P_2 activity (20). Here, we could hardly detect the activity of P_3.

Based on these results, we suggest the following model for the induction of *mazEF*-mediated cell death by thymine starvation. Previous research has shown that thymine starvation provokes DNA damage that involves a unique breaking or twisting of the chromosome into a configuration that defies all the repair/protective mechanisms of the cell (references 2 and 26 and references therein). We assume that such substantial damage to the DNA is responsible for the reduction of tran-

scription from the *mazEF* promoter P_2 (Fig. 4a). The effect of thymine starvation on the activity of this promoter may result from the direct starvation effect on P_2. Note that the promoter region of P_2 is particularly A:T rich: 20 nucleotides out of 26 (−20 to +6) are A or T (Fig. 4a). However, the interference with transcription from P_2 by thymine starvation may also be caused indirectly by the induction of ppGpp synthesis, known to inhibit the *mazEF* P_2 promoter (3), and/or by some specific protein(s) that could sense the damage to the DNA. Our experiments indicate that ppGpp might be involved. In these experiments, we compared the *mazEF*-dependent killing caused by trimethoprim in MC4100, which carries a defective *relA* gene (*relA1*) (3) and therefore does not synthesize ppGpp (9), to that of the *relA*$^+$ strain. We found that *mazEF*-dependent death appears in the *relA*$^+$ strain at 0.1 μg of trimethoprim/10^6 cells, while in the *relA1* strain it appears only at 0.3 μg of trimethoprim/10^6 cells (data not shown). Altogether, as a consequence of thymine starvation, prevention of the continuous expression of the labile antitoxin MazE results in cell death. Although we have shown that thymine starvation does not affect the activity of the *E. coli trpR* promoter (Fig. 4b), other promoters may be affected; these may include other genes involved in *mazEF*-mediated death.

Our results showing that thymine starvation can trigger a built-in death program provide new insight into an old enigma and may have implications for PCD in both prokaryotes and eukaryotes. (i) Until now, *mazEF*-directed PCD has been studied only in *E. coli*. However, this system is not unique to *E. coli* and is found on the chromosomes of other bacteria (12, 25). Nevertheless, TLD has been described in a wide range of microorganisms (2), suggesting the existence of analogous suicide modules and PCD not only in *E. coli* but in other bacteria as well. (ii) Our present results also increase the repertoire of antibiotics that operate in various bacteria by triggering a chromosomal suicide module. In previous work, it was shown that the *mazEF* suicide system in *E. coli* can be triggered by antibiotics like rifampin, chloramphenicol, and spectinomycin that inhibit transcription and/or translation (12, 27). Here, we extend this list to include trimethoprim and sulfonamides that inhibit folic acid metabolism and thereby cause TLD. (iii) Furthermore, our results may have implications for both mammalian TLD and cancer research. For decades, clinicians have used thymine starvation, leading to mammalian cell death, as a major method of cancer therapy. TLD is induced by treatment with the analogue 5-fluorouracil and with the folic acid antagonist methotrexate (29, 30). Thus, the knowledge gained from TLD as a trigger of bacterial PCD may be relevant to apoptosis in mammals and to cancer therapy.

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REFERENCES

1. **Ahmad, S. I.** 1980. Thymineless death in recombination deficient mutants of *Escherichia coli* K12. Z. Naturforsch. Teil C **35:**279–283.
2. **Ahmad, S. I., S. H. Kirk, and A. Eisenstark.** 1998. Thymine metabolism and thymineless death in prokaryotes and eukaryotes. Annu. Rev. Microbiol. **52:**591–625.
3. **Aizenman, E., H. Engelberg-Kulka, and G. Glaser.** 1996. An *Escherichia coli* chromosomal "addiction module" regulated by guanosine 3′,5′-bispyrophosphate: a model for programmed bacterial cell death. Proc. Natl. Acad. Sci. USA **93:**6059–6063.
4. **Angehrn, P., and R. Then.** 1973. Nature of trimethoprim-induced death in *Escherichia coli*. Arzneimittelforschung **23:**447–451.
5. **Bachmann, B. J.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54:**130–197.
6. **Barner, H. D., and S. S. Cohen.** 1957. The isolation and properties of amino acids requirement mutants of thymineless bacterium. J. Bacteriol. **74:**350–355.
7. **Bazill, G. W.** 1967. Lethal unbalanced growth in bacteria. Nature **216:**346–349.
8. **Benhar, I., and H. Engelberg-Kulka.** 1993. Frameshifting in the expression of the *E. coli trpR* gene occurs by bypassing of a segment of its coding sequence. Cell **72:**121–130.
9. **Cashel, M., D. R. Gentry, V. Z. Hernandez, and D. Vinella.** 1996. The stringent response, p. 1458–1496. *In* F. C. Neidhardt, R. Curtis III, J. L. Ingrahm, E. C. C. Lin, K. B. M. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. American Society for Microbiology, Washington, D.C.
10. **Cohen, S. S., and H. D. Barner.** 1954. Studies on the unbalanced growth in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **40:**885–893.
11. **Couturier, M., E. M. Bahassi, and L. Van Melderen.** 1998. Bacterial death by DNA gyrase poisoning. Trends Microbiol. **6:**269–275.
12. **Engelberg-Kulka, H., B. Sat, and R. Hazan.** 2001. Bacterial programmed cell death and antibiotics. ASM News **67:**617–624.
13. **Engelberg-Kulka, H., and G. Glaser.** 1999. Addiction modules and programmed cell death and antideath in bacterial cultures. Annu. Rev. Microbiol. **53:**43–70.
14. **Engelberg-Kulka, H., M. Reches, S. Narasimhan, R. Schoulaker-Schwarz, Y. Klemes, E. Aizenman, and G. Glaser.** 1998. *rexB* of bacteriophage lambda is an anti-cell death gene. Proc. Natl. Acad. Sci. USA **95:**15481–15486.
15. **Friedkin, M., and A. Kornberg.** 1957. The enzymatic conversion of deoxyridylic acid to thymidine acid and the participation of tetrahydrofolic acid, p. 609. *In* W. D. McElroy and B. Glass (ed.), A symposium on the chemical basis of heredity. Johns Hopkins University Press, Baltimore, Md.
16. **Gerdes, K., A. P. Gultyaev, T. Franch, K. Pederson, and N. D. Milkkelsen.** 1997. Antisense RNA-regulated programmed cell death. Annu. Rev. Genet. **19:**49–61.
17. **Gotfredsen, M., and K. Gerdes.** 1998. The *Escherichia coli relBE* genes belong to a new toxin-antitoxin gene family. Mol. Microbiol. **29:**1065–1076.
18. **Gunsalus, R. P., and C. Yanofsky.** 1980. Nucleotide sequence and expression of *Escherichia coli trpR*, the structural gene for the *trp* aporepressor. Proc. Natl. Acad. Sci. USA **77:**7117–7121.
19. **Hazan, R., B. Sat, M. Reches, and H. Engelberg-Kulka.** 2001. Postsegregational killing mediated by the P1 phage "addiction module" *phd-doc* requires the *Escherichia coli* programmed cell death system *mazEF*. J. Bacteriol. **183:**2046–2050.
20. **Marianovsky, I., E. Aizenman, H. Engelberg-Kulka, and G. Glaser.** 2001. The regulation of the *Escherichia coli mazEF* promoter involves an unusual alternating palindrome. J. Biol. Chem. **276:**5975–5984.
21. **Masuda, Y., and E. Ohtsubo.** 1994. Mapping and disruption of the *chpB* locus in *Escherichia coli*. J. Bacteriol. **176:**5861–5863.
22. **Masuda, Y., K. Miyakawa, Y. Nishimura, and E. Ohtsubo.** 1993. *chpA* and *chpB*, *Escherichia coli* chromosomal homologs of the *pem* locus responsible for stable maintenance of plasmid R100. J. Bacteriol. **175:**6850–6856.
23. **Metzger, S., I. B. Dror, E. Aizenman, G. Schreiber, M. Toone, J. D. Friesen, M. Cashel, and G. Glaser.** 1988. The nucleotide sequence and characterization of the *relA* gene of *Escherichia coli*. J. Biol. Chem. **263:**15699–15704.
24. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 205–210. Cold Spring Harbor Laboratory Press, Plainview, N.Y.
25. **Mittenhuber, G.** 1999. Occurrence of MazEF-like antitoxin/toxin systems in bacteria. J. Mol. Microbiol. Biotechnol. **1:**295–302.
26. **Nakayama, K., K. Kusano, N. Irino, and H. Nakayama.** 1994. Thymine starvation-induced structural changes in *Escherichia coli* DNA. Detection by pulse field gel electrophoresis and evidence for involvement of homologous recombination. J. Mol. Biol. **243:**611–620.
27. **Sat, B., R. Hazan, T. Fisher, H. Khaner, G. Glaser, and H. Engelberg-Kulka.** 2001. Programmed cell death in *Escherichia coli*: some antibiotics can trigger *mazEF* lethality. J. Bacteriol. **183:**2041–2045.
28. **Then, R., and P. Angehrn.** 1973. Sulphonamide-induced 'thymineless death' in *Escherichia coli*. J. Gen. Microbiol. **76:**255–263.
29. **Walder, S., R. Horowitz, X. Mao, and E. L. Schwartz.** 1996. Effect of interferon on 5-fluorouracil-induced perturbations in pools of deoxynucleotide triphosphates and DNA strand breaks. Can. Chemother. Pharmacol. **38:**529–535.
30. **Wei, X., H. L. McLeod, J. McMurrough, F. J. Gonzalez, and P. Fernandez-Salguero.** 1996. Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. J. Clin. Investig. **98:**610–615.
31. **Yarmolinsky, M. B.** 1995. Programmed cell death in bacterial populations. Science **267:**836–837.