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Anticipating an alkaline stress through the Tor phosphorelay system in *Escherichia coli*

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Summary

The *torCAD* operon encoding the TMAO reductase respiratory system is induced in the presence of TMAO by the two-component regulatory system TorS/TorR. The TorS sensor detects TMAO and transphosphorylates the TorR response regulator via a four-step phosphorelay. Once phosphorylated, TorR activates expression of the *torCAD* structural operon. In order to identify new genes regulated by the Tor regulatory system, we performed a genome-wide transcriptional analysis by using the DNA array technology. We identified seven new transcriptional units whose expression is modulated by the TorS/TorR phosphorelay system. One unit, *tnaLAB*, is positively regulated whereas the other six, *gadA*, *gadBC*, *hdeAB*, *hdeD*, *yhiE* and *yhiM*, are negatively regulated by this system. Interestingly, the products of some of these units seem to play a role in the survival of *E. coli* in conditions of extreme pH. The TnaA tryptophanase has been proposed to counteract alkaline stress, whereas the GadA and GadB glutamate decarboxylases and the HdeA and HdeB proteins are involved in the defence against acid stress. Our hypothesis is that the TorS/TorR phosphorelay triggers alkaline-stress defence to limit alkalinization resulting from the reduction of TMAO in alkaline TMA by the Tor respiratory system. The fact that a Δ*tnaLAB* mutant showed a dramatic decrease in survival as a result of TMAO respiration is in agreement with such a model. As regulation of these genes by the TorS/TorR system does not depend on pH modification but rather on the presence of TMAO, we propose that *E. coli* anticipates alkalinization of the medium due to TMA production by base-resistance gene activation and acid-resistance gene repression.

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Introduction

Escherichia coli is a facultative anaerobe. In the absence of oxygen, it is able to use different substrates as alternative electron acceptors like nitrate, nitrite, dimethyl sulphoxide or trimethylamine *N*-oxide (TMAO) (Gennis and Stewart, 1996). Trimethylamine *N*-oxide is a small compound widespread in nature that can be reduced to volatile TMA (trimethylamine) by the TMAO reductase respiratory system (Barrett and Kwan, 1985). The latter is composed of a *c*-type cytochrome, TorC, and a periplasmic molybdo-protein, TorA, which is the terminal TMAO reductase. The TorC and TorA proteins are encoded by the *torCAD* operon, the *torD* gene encoding a TorA specific chaperone (Méjean *et al*., 1994; Pommier *et al*., 1998).

The *torCAD* operon is expressed only in anaerobiosis and is positively controlled by the TorS/TorR two-component system in response to TMAO availability (Simon *et al*., 1994; Jourlin *et al*., 1996). Moreover, its expression is negatively regulated by the apocytochrome TorC (Ansaldi *et al*., 1999). The TorS protein is an unorthodox transmembrane sensor composed of a periplasmic N-terminal domain and a large cytoplasmic C-terminal domain containing three sites of phosphorylation (His443, Asp723 and His850) (Jourlin *et al*., 1996). TorS can be considered as one of the most complex sensors for several reasons. First, the TorS sensor detects at least two signals with antagonist action: TMAO that activates it and apoTorC that inhibits it. Indeed, the periplasmic domain of TorS probably detects the presence of TMAO as a small deletion in it (mutant TorS726) mimics the presence of TMAO and allows constitutive expression of the *tor* operon (Jourlin *et al*., 1996). Moreover, we recently showed that this periplasmic detector region can also bind the apocytochrome TorC (Gon *et al*., 2001). Second, TorS transphosphorylates the TorR response regulator via a four-step phosphorelay (His443 → Asp723 → His850 → Asp53 of TorR) (Jourlin *et al*., 1997). Third, TorS dephosphorylates TorR-P when TMAO is exhausted. This dephosphorylation

is performed by a reverse phosphorelay: TorR Asp53 → His850 → Asp723 (Ansaldi *et al*., 2001). Despite this complexity, there is only one known target for the TorR protein: the *torC-torR* intergenic region. Binding of TorR to decameric repeats in this intergenic region activates transcription of the *torCAD* operon and downregulates *torR* expression (Simon *et al*., 1995; Ansaldi *et al*., 2000).

The availability of gene arrays permits the simultaneous study of the expression of almost all genes in an organism. This approach has been used for the study of both eukaryotic and prokaryotic systems. In particular, it has been successfully used in studying transcriptional profiles of *E. coli* grown in various conditions (Tao *et al*., 1999; Arfin *et al*., 2000; Brocklehurst and Morby, 2000; Khodursky *et al*., 2000; Hommais *et al*., 2001; Pomposiello *et al*., 2001; Zheng *et al*., 2001). To identify new targets of the TorS/TorR regulatory system, we compared genome-wide expression profiles of a wild-type strain grown either in the presence or absence of TMAO and of strains with different genetic backgrounds grown in the same conditions. We identified a small number of new genes potentially regulated by the TorS/TorR system. Interestingly most of these genes are involved in survival to extreme pH. Therefore, the Tor regulatory system not only induces the TMAO reductase operon but also allows the cell to counteract alkalinization resulting from TMA production.

Results

Analysis of transcriptional profiles

In order to identify new genes potentially regulated by the Tor regulatory system, we carried out macroarray experiments by using the Panorama gene arrays (Sigma-Genosys) which contain most of the genomic ORFs of *E. coli*. We compared genome-wide expression profiles obtained in an inducing condition, in which the TorR protein is active, and in a non-inducing condition (also called reference condition), in which the TorR protein is inactive.

Initially we compared the MC4100 strain, which is wild-type for the *tor* locus, grown anaerobically in L-broth in the absence (reference condition) and in the presence (inducing condition) of TMAO. The ratio of expression between the inducing condition and the reference condition was calculated for every gene (see *Experimental procedures*) and expressed in the logarithmic form. We considered that a gene is activated if its log ratio is greater than the mean of the log ratios plus two standard deviations and that a gene is repressed if its log ratio is lower than the mean of the log ratios minus two standard deviations. Applying these criteria, we found that around 200 genes were differentially expressed: most of these genes were activated in the presence of TMAO (86%) whereas the others were repressed. These genes belong to two categories: (i) genes regulated by the TorS/TorR regulatory system and (ii) genes regulated by a Tor-independent effect of TMAO.

In order to eliminate any Tor-independent effects of TMAO and therefore to identify the TorS/TorR-regulated genes, we performed three additional experiments using specific genetic constructions. In one experiment, we compared a *torR* strain grown anaerobically in the presence of TMAO (reference condition) to a wild-type strain also grown in the presence of TMAO (inducing condition). In the two other experiments, we compared strains grown anaerobically in the absence of TMAO: a *torS*$^+$ strain (reference condition) and two *torS* constitutive strains which are able to express the *tor* operon in the absence of TMAO (inducing condition). The *torS* alleles were either plasmid-born or chromosomal.

Interestingly, very few genes were identified that showed differential expression in all four experiments: two genes are potentially activated and six are potentially repressed by the Tor regulatory system in the presence of TMAO (Table 1 and Fig. 1; for complete results, see *Supplementary material*). Even if the expression of these genes varied in a similar way in all four experiments, it is noteworthy that, for an unknown reason, the ratios showed significant differences.

The macroarray-identified genes

The gene exhibiting the highest averaged ratio of activation (i.e. 31.9) is the *torC* gene. This result confirms that the *tor* operon is strictly controlled by the TorS/TorR system. Most of the strains used for the macroarray experiments contain a chromosomal wild-type copy of the *torA* and the *torD* genes, nevertheless neither *torA* nor *torD* was found as activated in the corresponding experiments. Although the fact that not every gene of an operon can be visualized is surprising, it seems to be a general phenomenon observed in DNA array experiments (Barbosa and Levy, 2000; Khodursky *et al*., 2000; Zimmer *et al*., 2000).

The second activated gene is *tnaA* with an averaged ratio of 15.4. This gene is part of the *tnaLAB* operon. The *tnaL* gene encodes a leader peptide involved in the tryptophan-induced transcription antitermination, the *tnaA* gene encodes the tryptophanase and the *tnaB* gene encodes a low-affinity tryptophan permease (Deeley and Yanofsky, 1981; Edwards and Yudkin, 1982; Stewart and Yanofsky, 1985; 1986). The *tnaL* and *tnaB* genes were also found to be activated but the activation ratio is above the applied threshold in only three of the four macroarray experiments for the *tnaL* gene and in only one for the *tnaB* gene.

The genes showing the greatest averaged repression ratio are the *hdeA* and *hdeB* genes (i.e. around 11). These genes form an operon but little is known about them except that they encode periplasmic proteins (Yoshida

Table 1. Genes differentially expressed in all macroarray experiments.

Genes	Description	Ratio[a] (Log ratio) Exp1	Exp2	Exp3[b]	Exp4	Mean ratio
Activated						
torC	Cytochrome *c*-type protein TorC	5.8 (0.765)	6.8 (0.835)	110.5 (2.043)	4.3 (0.630)	31.9
tnaA	Tryptophanase	14.8 (1.171)	32.1 (1.507)	12.5 (1.095)	2.3 (0.362)	15.4
Repressed						
hdeB	Unknown function	3.8 (0.582)	10 (0.998)	25.5 (1.407)	3.9 (0.594)	10.8
hdeA	Unknown function	5.8 (0.763)	5.1 (0.704)	28 (1.448)	2.7 (0.425)	10.4
yhiE	Unknown function	5.1 (0.711)	4.2 (0.619)	19.8 (1.297)	3.6 (0.553)	8.2
gadA	Glutamate decarboxylase	5.1 (0.710)	8.3 (0.918)	3.1 (0.487)	3.3 (0.523)	4.9
hdeD	Unknown function	3.5 (0.544)	5 (0.703)	5.9 (0.769)	4 (0.597)	4.6
yhiM	Unknown function	5.7 (0.759)	2.7 (0.427)	3.6 (0.552)	2.5 (0.400)	3.6

a. The ratio given for the activated genes correspond to the ratio of expression between the inducing condition and the reference condition, whereas, for convenience, the ratio given for the repressed genes correspond to the ratio of expression between the reference condition and the inducing condition.
b. In experiment 3, both strains contain a multicopy plasmid carrying a *torCA-lac* fusion and the *torSTR* gene cluster, which might explain the high ratio obtained in particular for the *torC* gene.

et al., 1993). It is interesting to note that two genes, *hdeD* and *yhiE*, that are located close to the *hdeAB* operon were also found to be repressed (repression ratio of 4.6 and 8.2, respectively). The *hdeD* gene is upstream and divergently transcribed from the *hdeAB* operon, whereas the *yhiE* gene is downstream and transcribed in the same direction as the *hdeD* gene.

The *gadA* gene was also found to be repressed. This gene encodes a glutamate decarboxylase. In *E. coli*, there are two homologous glutamate decarboxylases, GadA and GadB. The genes encoding these enzymes are highly homologous (98% identity at the nucleotide level) but map at different loci on the chromosome (Smith *et al*., 1992). The *gadB* gene is part of an operon, the *gadBC* operon (Hersh *et al*., 1996; De Biase *et al*., 1999). The *gadB* gene was also found to be repressed but the repression ratio is above the applied threshold in only two of the four macroarray experiments. Considering the high degree of homology between these two genes, cross-hybridization cannot be ruled out. So either the *gadA* gene or the *gadB* gene, or even both genes could be repressed by the Tor regulatory system in the presence of TMAO. The last gene which was found to be repressed with an averaged ratio of 3.6 is the *yhiM* gene of unknown function.

TMAO- and TorR-dependent regulation of the new identified transcriptional units

In order to verify that the genes identified by the macroarray experiments were regulated in response to the presence of TMAO, we constructed plasmids with transcriptional fusions. The promoter of the potentially activated unit (*tnaLAB*) and of all potentially repressed units (*hdeAB*, *hdeD*, *gadA*, *yhiE* and *yhiM*) were cloned upstream of the promoterless *lacZ* gene. To discriminate between the *gadA* gene and the *gadB* gene, we made an additional construction with the promoter of the *gadB* gene. The activities of the seven promoters were then estimated by measuring the β-galactosidase activity in the plasmid-containing MC4100 strain grown anaerobically to exponential phase in L-broth in the absence and presence of TMAO.

Figure 2 shows that the expression of the *tnaA-lacZ* fusion is 3.5-fold higher in the presence of TMAO than in its absence. The expression of the fusion was also measured in another condition of anaerobic respiration, that is in the presence of nitrate. We showed that the level of expression is nearly four-times higher in the presence of TMAO than in the presence of nitrate (data not shown). On the contrary, the expression of the *gadA-lacZ*, *hdeA-lacZ*, *hdeD-lacZ*, *yhiE-lacZ* and *yhiM-lacZ* fusions is higher in the absence of TMAO than in its presence (two- to fourfold). These results are consistent with those of the macroarray experiments (Table 1 and Fig. 1) and confirm that *tnaLAB* is activated in the presence of TMAO whereas *gadA*, *hdeAB*, *hdeD*, *yhiE* and *yhiM* are repressed. Moreover the *gadB-lacZ* fusion shows a regulation similar to that of the *gadA-lacZ* fusion, i.e. an higher expression in

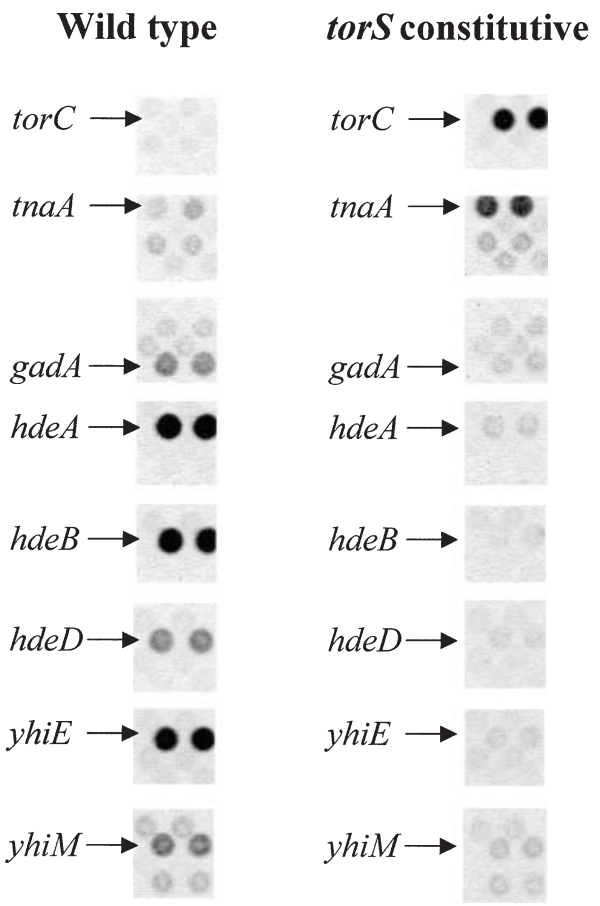

Fig. 1. Close-ups of expression profiles of *Escherichia coli* strains with wild-type and constitutive *torS* alleles. A pair of DNA arrays was hybridized with ^{33}P-labelled cDNA prepared from total RNA of wild-type *torS* strain (MC4100/pMCP3, left panel) and constitutive *torS* strain (MC4100/pMCP13, right panel). The positions of the genes of interest are indicated by arrows.

the absence of TMAO (3.5-fold). Therefore both *gadA* and *gadB* are repressed in the presence of TMAO.

In order to determine if the regulation of these seven units in the presence of TMAO was TorR-dependent, we introduced the plasmids containing the promoter fusions in a *torR* strain (LCB621). The β-galactosidase activities were then measured in the presence and in the absence of TMAO. Figure 2 shows that, in the *torR* strain, the *tnaA-lacZ* fusion is poorly activated by the presence of TMAO. For the other fusions, the repression in the presence of TMAO is almost completely lost in the *torR* strain. Therefore, the regulation of these seven units in the presence of TMAO is mainly TorR-dependent, although the action of TorR could be either direct or indirect.

Binding of TorR to the tna *operon promoter*

The TorR protein induces the *torCAD* operon directly by binding to the *torC* control region (Simon *et al*., 1995). To determine whether the TorR protein also induces expression of the *tna* operon directly by binding to the promoter region, we carried out DNase I footprinting analysis. A DNA fragment encompassing the *tna* promoter was generated by PCR and then end-labelled on one strand. This fragment was incubated with increasing concentration (0–10 μM) of TorR and then treated with DNase I. Two regions of about 18 bp each were protected in the presence of TorR (Fig. 3A). A DNase I hypersensitive site appeared at position −91, suggesting that binding of TorR to DNA induces local distortion.

The two protected regions are, respectively, centred around positions −55 (region I) and −100 (region II), and are A/T rich. Although neither palindromic sequences nor direct repeats are present in these regions, it is noteworthy that a sequence matching seven of the 10 bases of the *tor* box (CTGTTCATAT; Simon *et al*., 1995) is found in region I (CGATTCACAT) (Fig. 3B). Another interesting point is the overlap between the TorR-protected region I and a cAMP-CRP binding site located at position −60 (Botsford and DeMoss, 1971; Stewart and Yanofsky, 1985). This suggest that, depending on the growth conditions, either the TorR protein or the cAMP-CRP complex binds to the *tna* promoter. This result strongly suggests that TorR induces *tnaLAB* operon expression directly by binding to two regions of the promoter and that this binding leads to DNA bending.

Resistance to extreme pH: a common theme

Interestingly, the products of four of the new TMAO-regulated units could be involved in the survival of *E. coli* in conditions of extreme pH. Indeed, the TnaA tryptophanase was shown to be induced at high pH (Blankenhorn *et al*., 1999; Stancik *et al*., 2002). This enzyme degrades L-tryptophan to indole, pyruvic acid and ammonia (McFall and Newman, 1996). In anaerobiosis, pyruvic acid could then be transformed in formic and acetic acids. It was then proposed that TnaA could reverse alkalinization by producing acidic products (Blankenhorn *et al*., 1999).

On the contrary, the GadA and GadB glutamate decarboxylases contribute to the acid survival of *E. coli* (Castanié-Cornet *et al*., 1999; De Biase *et al*., 1999). These enzymes catalyse the conversion of glutamate to γ-aminobutyrate (GABA), which is released through a GadC-dependent excretion mechanism, and CO_2. During this process, an H^+ ion is consumed which would raise the pH. The HdeA and HdeB proteins could also be implicated in the acid-stress defence. Indeed, the deletion of the *hdeA* gene abolishes *E. coli* acid resistance (Gajiwala and Burley, 2000). Moreover the HdeA protein was shown to prevent aggregation of denatured proteins at low pH and

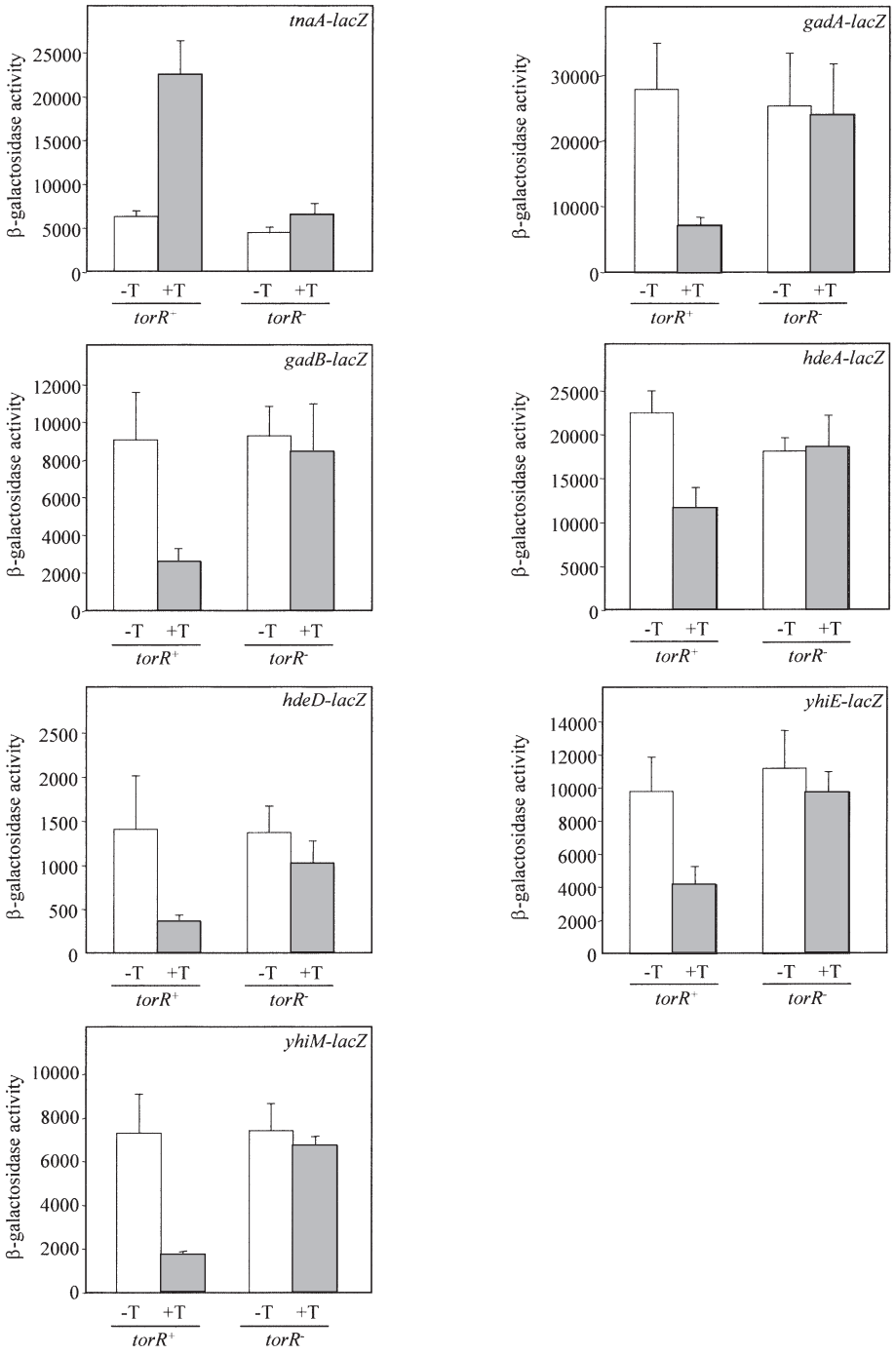

Fig. 2. β-galactosidase activities of transcriptional fusions. Wild-type and *torR* mutant strains carrying plasmid-born transcriptional fusions were grown anaerobically on L-broth in the absence (–T) or presence (+T) of 10 mM TMAO. β-galactosidase activity, expressed in Miller units, was measured on whole cells harvested during exponential phase. Error bars are indicated.

was proposed to be a chaperone-like protein that supports acid resistance in enteric bacteria (Gajiwala and Burley, 2000).

Therefore the Tor regulatory system appears, in the presence of TMAO, to activate a system allowing survival at high pH and to repress systems allowing survival at low pH. The physiological reason of this phenomenon could be that TMA, the product resulting from TMAO reduction, is an alkaline molecule (Barrett and Kwan, 1985) with a pK_a of 9.87. If TMAO reduction results in alkalinization of

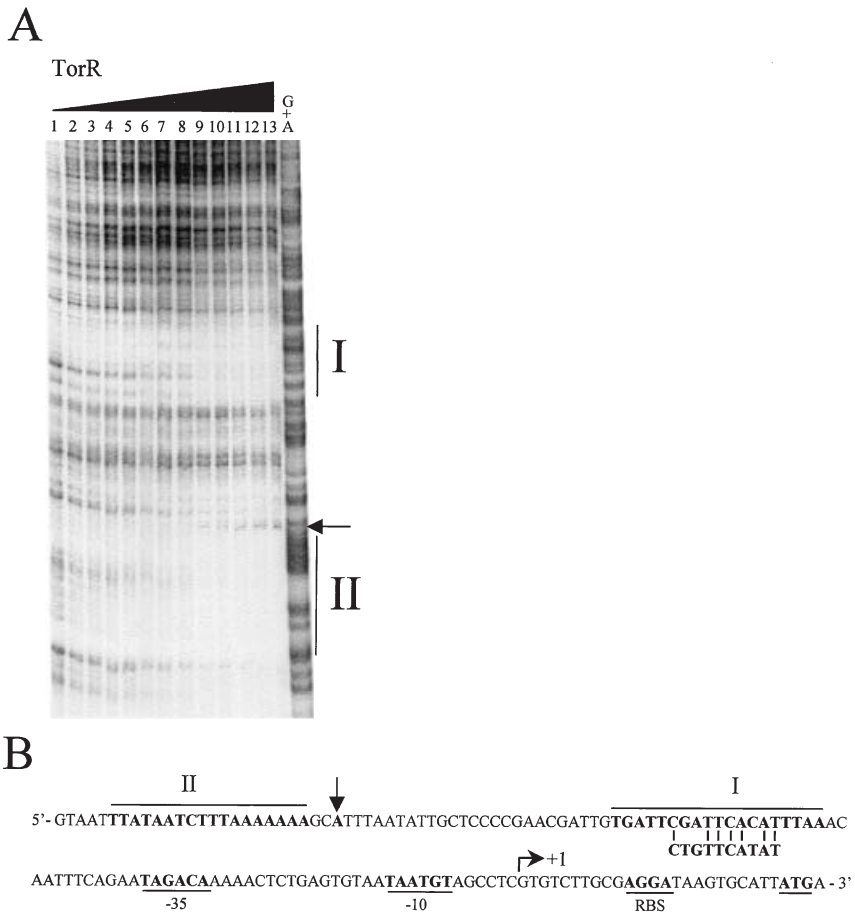

Fig. 3. Analysis of TorR binding to the *tnaLAB* promoter region by DNase I footprinting experiments.
A. The labelled DNA fragment, prepared as described in *Experimental procedures*, was digested with 0.66 units of DNase I in the presence of various concentrations of purified TorR protein: Lane 1, no protein; lane 2, 0.75 μM; lane 3, 1 μM; lane 4, 1.25 μM; lane 5, 1.5 μM; lane 6, 2 μM; lane 7, 2.5 μM; lane 8, 3 μM; lane 9, 4 μM; lane 10, 5 μM; lane 11, 6 μM; lane 12, 7 μM and lane 13, 10 μM. The G + A sequencing ladder is indicated. Vertical bars indicate the protected regions and the arrow shows a DNase I hypersensitive site.
B. Nucleotide sequence of the *tna* promoter. The regions protected by TorR are overlined. Alignment of region I with high affinity *tor* box is indicated. The −10 and −35 regions and the ribosome binding site are underlined. The +1 arrow corresponds to the transcription start site and the vertical arrow indicates DNase I hypersensitive site.

the medium, then *E. coli* must protect itself by activating a mechanism of defence. Production of the tryptophanase could allow alkalinization to be limited, whereas repression of the glutamate decarboxylases would prevent an even higher increase of the pH.

Tryptophanase is involved in the survival of E. coli *to alkaline stress resulting from TMAO respiration*

As TMAO reduction occurs in the periplasm, we wondered whether the pH of the medium could be affected by TMA production. Consequently we followed pH modification during anaerobic growth of strain MC4100 in the absence and in the presence of TMAO until late stationary phase. Growth was performed in L-broth medium containing less yeast extract than usual to test the effect of tryptophan addition (see *Experimental procedures*) (Wang *et al*., 2001). As shown in Fig. 4, the pH of the medium was around 6.8 when MC4100 was grown in the absence of TMAO (Fig. 4A) whereas, in the presence of TMAO, the pH increased rapidly to reach a maximal value around 8.6 (Fig. 4C). Therefore growth in the presence of TMAO actually led to alkalinization of the medium.

We then looked at the survival of the MC4100 strain. The number of viable cells present in the culture was estimated by counting the cells still able to form colonies. A constant number of viable cells is observed in stationary phase for the MC4100 strain grown in the absence of TMAO (Fig. 4B). On the contrary, the same strain grown in the presence of TMAO shows a dramatic decrease of survival since there is around 26-fold less viable cells when the strain is grown in the presence of TMAO than when it is grown in the absence of TMAO (Fig. 4D).

To study the possible involvement of the tryptophanase in cell survival at high pH, we performed the same experiments but in the presence of 1 mM tryptophan. Figure 4A and B shows that, for the cells grown in the absence of TMAO, the presence of tryptophan has no effect on either the pH or the survival. However, for the cells grown in the presence of TMAO, it is interesting to note that the pH of the medium increases more slowly (Fig. 4C). In particular, after 24 h of growth, the pH is 0.6 unit lower when tryptophan is present. Strikingly, in the presence of tryptophan, the cell survival is no longer affected by the TMAO reduction (Fig. 4D).

To confirm that the tryptophanase is directly implicated in this phenomenon, we constructed a mutant deleted of the whole *tna* operon. This mutant was then grown in the

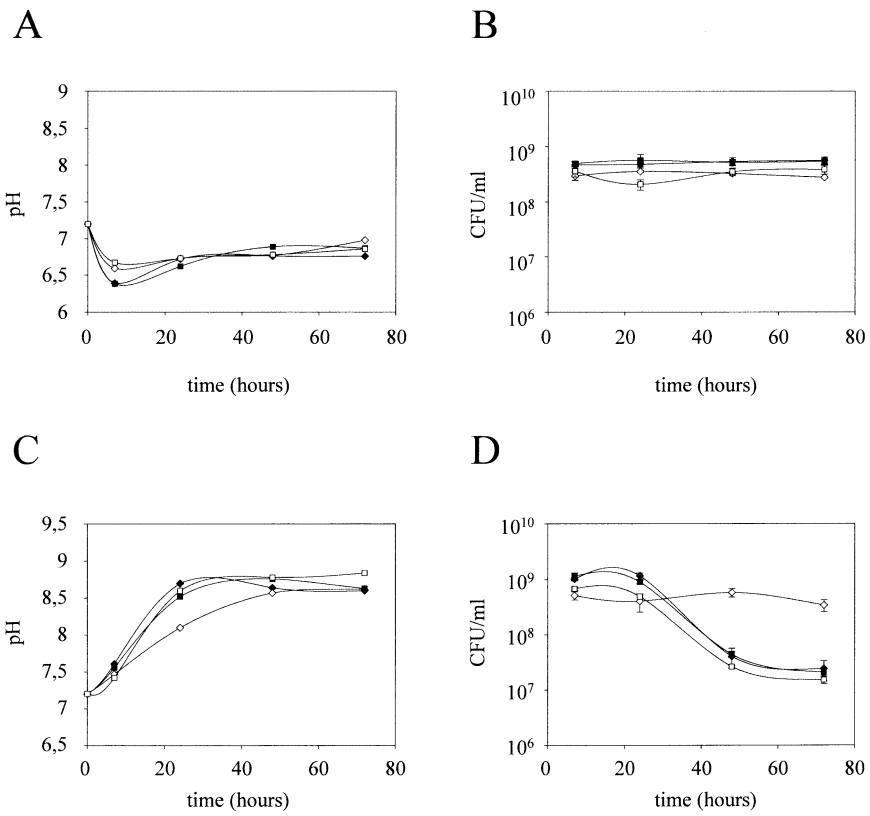

Fig. 4. pH changes and the cell survival of wild-type and *tna* mutant strains grown in the absence and presence of TMAO. Strains were grown as specified (see *Experimental procedures*) in the absence (A and B) and in the presence (C and D) of 40 mM TMAO. The pH of the medium was measured along growth (A and C). The number of viable cells is expressed as the number of bacteria still able to form colonies (B and D). The MC4100 strain is represented by diamonds and the *tna* mutant (LCB430 strain) by squares. Strains were grown in the absence (closed symbols) or in the presence of 1 mM tryptophan (open symbols). Error bars representing the 95% confidence intervals obtained from three experiments are indicated for the survival assays.

same condition as strain MC4100 and the pH of the medium as well as the cell survival was followed (Fig. 4). In the *tna* mutant, the pH increases rapidly in the presence of TMAO and a dramatic decrease of survival is consequently observed even in the presence of tryptophan. These results demonstrate that the tryptophanase system allows *E. coli* to survive to the alkalinization of the medium due to TMAO reduction.

Regulation by the TorS/TorR system depends on the presence of TMAO and not on pH

Some of the new transcriptional units regulated by the Tor phosphorelay system are already known to be regulated in response to others signals. In particular, three of them were shown to be regulated in response to pH. A two-dimensional gel electrophoresis approach revealed that TnaA was induced about 2.5-fold in cells grown anaerobically in LB medium buffered at pH 9, compared to the response at pH 4.5 (Blankenhorn *et al*., 1999). Another study carried out on aerobic cultures showed that a *tnaA-lacZ* fusion is greatly induced at pH 9 compared to pH 7, but the difference of expression is low between pH 7 and pH 8 (Stancik *et al*., 2002). The *gadA* and *gadB* genes are induced by acid pH (around 5) (Castanié-Cornet *et al*., 1999; De Biase *et al*., 1999; Castanié-Cornet and Foster, 2001). It was also shown that the *gad* genes and the *hdeAB* operon are induced by acetate (Arnold *et al*., 2001; Kirkpatrick *et al*., 2002).

As some of these units are known to be regulated in response to pH, it could be possible that the differences of expression we observed were due to the increase of pH resulting from TMAO reduction rather than to the presence of TMAO *per se*. Several results strongly suggest that this is not the case. First, the cells used for the macroarray experiments and for the transcriptional fusion measurements were grown to exponential phase, that is around 3 h of growth. In these conditions, the pH of the medium never exceeded 7.2. Second, two of the macroarray experiments were carried out from cells grown in the absence of TMAO (*torS* wild type versus *torS* constitutive). Although no alkalinization from TMA production was pos-

sible, we observed a great difference of spot intensities for the concerned units (Fig. 1). In order to parallel the fourth macroarray experiment and to validate our assumption, all the promoter fusions were introduced in strain LCB726 (*torS* constitutive) as well as in strain LCB620 (*torS* wild type). The β-galactosidase activities were measured in the two strains grown anaerobically in the absence of TMAO (Fig. 5). Although both strains exhibited a similar pH (6.4 ± 0.1), the activation of the *tnaA-lacZ* fusion and the repression of the other fusions were observed in strain LCB726. These results are consistent with our hypothesis that regulation of these genes by the TorS/TorR system depends on the presence of TMAO rather than on pH modification. Therefore we propose that the modulation of the expression of these genes by the TorS/TorR system constitutes an anticipatory response to the alkalinization resulting from TMAO reduction to TMA.

Discussion

Our study has revealed that the TorS/TorR regulatory system regulates the expression of only a few genes. It is quite intriguing that, except the *torCAD* operon, these genes are apparently not related to any respiratory systems. In particular, none of the TorS/TorR target genes encode proteins involved in the electron transfer to the Tor respiratory system, such as primary dehydrogenases or cytochromes. Similarly, the genes encoding proteins

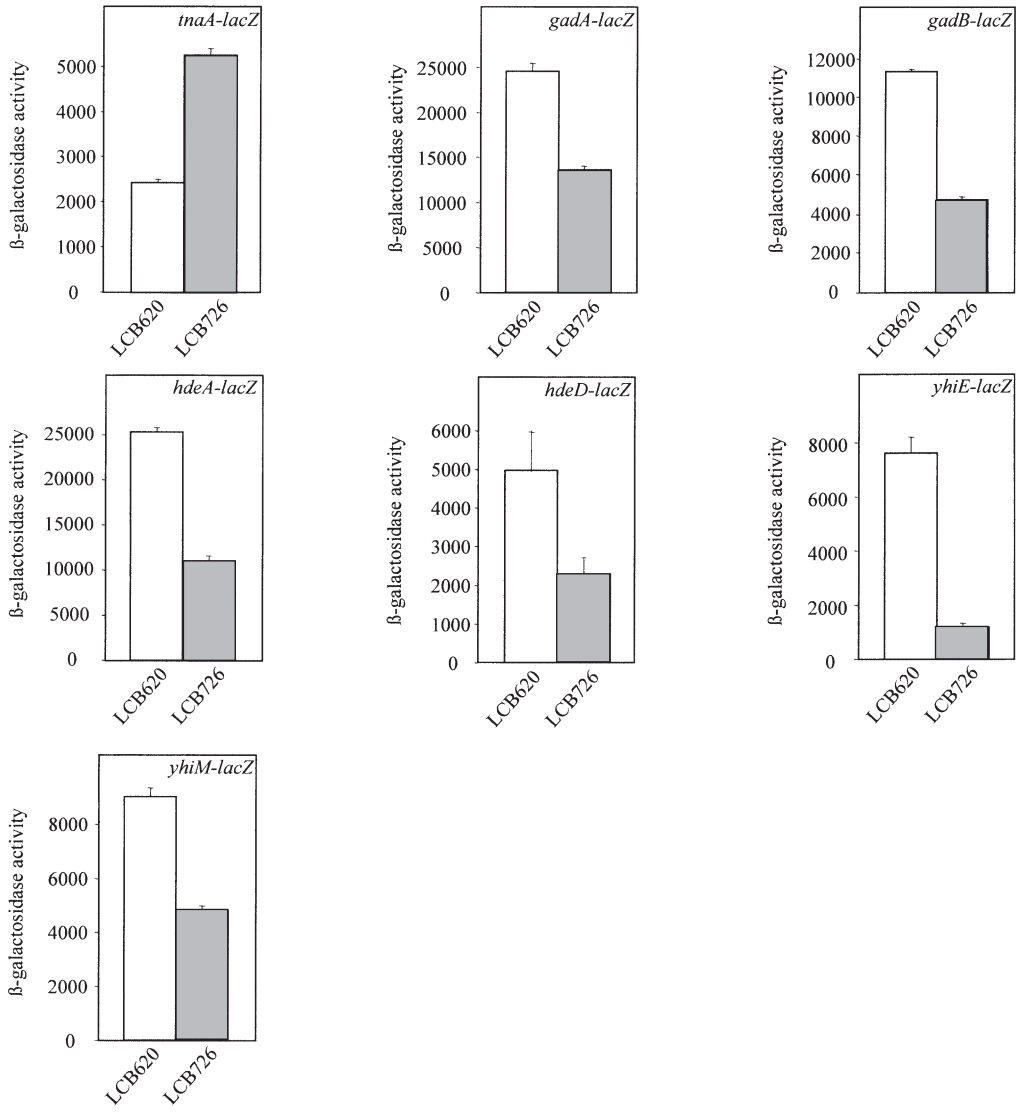

Fig. 5. β-galactosidase activities of transcriptional fusions in *torS* wild-type and in *torS* constitutive strains. LCB620 (wild-type *torS* allele) and LCB726 (constitutive *torS* allele) strains carrying plasmid-born transcriptional fusions were grown anaerobically on L-broth. β-galactosidase activity, expressed in Miller units, was measured on whole cells harvested during exponential phase. Error bars are indicated.

involved in the *c*-type cytochrome maturation process or in haem biosynthesis do not seem to be regulated by the TorS/TorR regulatory system. This last result is in agreement with previous reports showing that the *ccm* operon is not regulated by TMAO (Grove *et al*., 1996; Tanapongpipat *et al*., 1998). Genes involved either in the maturation or in the translocation of the molybdo-protein TorA are apparently not regulated by the TorS/TorR regulatory system either.

It is noteworthy that, during the completion of this paper, a transcriptome analysis of all two component systems of *E. coli* was published (Oshima *et al*., 2002). The genes showing altered expression in the Δ*torSTRCAD* mutant, in this study, were different from those that proved to be regulated by the TorS/TorR system in the presence of the inducer TMAO. This result is probably not surprising because the study of Oshima *et al*. (2002) was performed from cultures grown in aerobic condition which is not the condition of activation of the TorS/TorR system.

In conclusion, TorS/TorR is a system specifically dedicated to TMAO respiration rather than a global regulatory system in *E. coli*. Indeed, in the presence of TMAO, the TorS/TorR system not only induces the *torCAD* operon but also activates an alkaline-stress defence and represses acid-stress defences in order to limit alkalinization of the medium that will result from TMA production. Based on this assumption, we postulate that the *hdeD*, *yhiE* and *yhiM* genes, which were repressed in the presence of TMAO, could also be involved in a mechanism of acid-stress defence. This hypothesis is reinforced by the fact that the *yhiE* and the *hdeD* genes are close to the *hdeAB* operon. Moreover, a recent study showed that YhiE is indeed involved in acid resistance (Tucker *et al*., 2002). Therefore, these genes might constitute a locus involved in the resistance to acid stress.

Experimental procedures

Strains, plasmids and growth conditions

The strains and plasmids used in this study are listed in Table 2. Bacteria were grown in Luria–Bertani (LB) medium. For the survival assays, the cells were grown in L-broth containing 3 g l^{-1} of yeast extract instead of the 5 g l^{-1} normally used (Wang *et al*., 2001). All the media were unbuffered. When necessary antibiotics were added at the following concentrations: ampicillin, 50 μg ml^{-1}, streptomycin, 100 μg ml^{-1} and chloramphenicol, 25 μg ml^{-1}. For some experiments, TMAO and tryptophan were added as specified.

RNA preparations

Overnight cultures were diluted in L-broth in order to obtain the same initial optical density for all strains. The strains were then grown anaerobically. Cells were harvested during exponential phase, that is after about 3 h of growth. Cells were pelleted by centrifugation at 4°C and rapidly processed to isolate RNA using the High pure RNA isolation kit from Roche Diagnostics with the slight following modification: the DNase I digestion step was carried out twice in order to diminish the quantity of contaminating DNA. RNA was concentrated after

Table 2. Strains and plasmids.

Strain/Plasmid	Relevant characteristics	Source/reference
Strain		
MC4100	*araD139* Δ*(lacIPOZYA-argF)* U169 *rpsL thi*	M.J. Casadaban
LCB621	MC4100 *torR49*::miniTn*10*	Pascal *et al*. (1991)
LCB620	MC4100 *torA8*::MudII1734 (*torA′-′lacZYA*)	Méjean *et al*. (1994)
LCB726	LCB620 *tor*S726 (constitutive *torS* allele)	Jourlin *et al*. (1996)
LCB430	MC4100 Δ*tnaLAB*	This work
Plasmid		
pMCP3	MudII4042 carrying 9kb insert upstream from *torA′-′lacZYA* fusion	Jourlin *et al*. (1996)
pMCP13	Mutant derivative of pMCP3 (constitutive *torS* allele)	Jourlin *et al*. (1996)
pGE593	*lacZ* fusion vector with a colE1 origin of replication	Eraso and Weinstock (1992)
pPgadA	Promoter region of *gadA* inserted into pGE593 (position −390 to +178 relative to the ATG of *gadA*)	This work
pPgadB	Promoter region of *gadBC* inserted into pGE593 (position −353 to +121 relative to the ATG of *gadB*)	This work
pPhdeA	Promoter region of *hdeAB* inserted into pGE593 (position −360 to +121 relative to the ATG of *hdeA*)	This work
pPhdeD	Promoter region of *hdeD* inserted into pGE593 (position −236 to +143 relative to the ATG of *hdeD*)	This work
pPtnaA	Promoter region of *tnaLAB* inserted into pGE593 (position −223 to +340 relative to the ATG of *tnaL*)	This work
pPyhiE	Promoter region of *yhiE* inserted into pGE593 (position −354 to +205 relative to the ATG of *yhiE*)	This work
pPyhiM	Promoter region of *yhiM* inserted into pGE593 (position −244 to +163 relative to the ATG of *yhiM*)	This work

ethanol precipitation and resuspension in DEPC-treated water. The integrity of the RNA preparations was verified by electrophoresis on agarose gel. Absence of contaminating DNA was checked by PCR reaction.

Probe synthesis

Probes were generated by standard cDNA synthesis. Briefly, RNA was first hybridized with either the specific primer set (from Sigma-Genosys) or hexameric primers (Gibco-BRL). Then dATP, dTTP and dGTP (final concentrations, 0.33 mM each) were added together with 20 μCi of [α-^{33}P]-dCTP (3000 Ci mmol^{-1}) and 200 units of Superscript II (Gibco-BRL). The reaction was incubated 1 h at 42°C and stopped by 15 min at 70°C. Unincorporated nucleotides were removed by using the QIAquick nucleotide removal kit (QIAgen).

Hybridization

The DNA arrays used in this study were the Panorama *E. coli* gene arrays produced by Sigma-Genosys. The hybridization of the purified labelled cDNA to the arrays was performed in roller bottles according to the manufacturer's instructions. Briefly, arrays were prehybridized in 5 ml of hybridization solution (5 × SSPE, 2% sodium dodecyl sulphate, 1 × Denhardt's reagent, 100 μg ml^{-1} of sheared salmon sperm DNA) for at least one hour at 65°C in an hybridization oven. Then the cDNA probes were denatured in 3 ml of hybridization solution at 95°C and the arrays were hybridized with these solutions for 18 h at 65°C. The arrays were washed with the specified washing buffer (0.5 × SSPE, 0.2% SDS) three times for 3 min each at room temperature and three times for 20 min each at 65°C. The arrays were then wrapped in saran and exposed to a PhosphorImager screen (Molecular Dynamics) for 1–3 days.

Quantification and analysis of the arrays

The exposed PhosphorImager screens were scanned at a 50-μm pixel resolution on a STORM 820 PhosphorImager (Molecular Dynamics). Quantification was performed by using the ArrayVision software (Imaging Research). The intensity of each spot was corrected by subtracting the background of the membrane which was determined in an empty space outside the concerned field. The corrected intensity of each spot was then normalized by using the spots of genomic DNA present in the same field. The data were exported in Microsoft Excel and the pixel values for the duplicate spots were averaged. The expression ratio for each gene was calculated as the ratio between the intensity in the inducing condition and the intensity in the reference condition.

Two criteria were applied to the data. First, the normalized intensities of the 294 duplicated spots that did not contain DNA were averaged and only the genes that exhibited an intensity higher than this averaged value plus two standard deviations in at least one condition were further analysed. Second, only the genes for which the log of the expression ratio was greater than the mean of the log ratios plus or minus two standard deviations were considered activated or repressed, respectively.

Design of the macroarray experiments

For the first experiment, the MC4100 strain was grown in L-broth in the absence (reference condition) and in the presence (inducing condition) of 10 mM TMAO. Cells were harvested during exponential phase when the optical density was between 0.4 and 0.5. The reverse transcription was performed with hexameric primers.

For the second experiment, the LCB621 strain (reference condition) and the MC4100 strain (inducing condition) were grown in L-broth in the presence of 10 mM TMAO. Cells were harvested during exponential phase when the optical density was around 0.7. The reverse transcription was performed with the specific primer set.

For the third experiment, the MC4100 strain containing either the pMCP3 plasmid (reference condition) or the pMCP13 plasmid (inducing condition) were grown in L-broth containing chloramphenicol in the absence of TMAO. Cells were harvested during exponential phase when the optical density was between 0.2 and 0.3. The reverse transcription was performed with hexameric primers.

For the fourth experiment, the LCB620 strain (reference condition) and the LCB726 strain (inducing condition) were grown in L-broth in the absence of TMAO. Cells were harvested during exponential phase when the optical density was between 0.4 and 0.7. The reverse transcription was performed with hexameric primers.

Construction of plasmid-born transcriptional fusions

The cloning strategy was as followed. For the activated unit, the cloned region comprised around 500 nucleotides upstream of the translation initiation site. For the repressed units, the cloned region comprised around 400 nucleotides upstream of the translation initiation site and around 150 nucleotides downstream of it. DNA fragments were amplified from chromosomal DNA by PCR using an upstream primer containing an *Eco*RI site and a downstream primer containing a *Bam*HI site. The PCRs were performed with the Expand High Fidelity PCR system (Roche Diagnostics). The PCR products were purified using the QIAquick PCR purification kit (QIAgen). After digestion with *Eco*RI and *Bam*HI, these fragments were then ligated using the T4 DNA ligase (Biolabs) to the pGE593 plasmid previously linearized with *Eco*RI and *Bam*HI. All plasmids were checked by PCR with the upstream primer of the insert and a *lacZ* primer complementary to the *lacZ* sequence of pGE593.

β-Galactosidase assays

β-Galactosidase activities were measured on whole cells by the method of Miller (1972) after anaerobic growth at 37°C when the cultures were in exponential phase. Values represent the averages of at least three independent experiments.

The activities of the plasmid-born promoter fusions in the LCB620 and LCB726 strains correspond to the value measured on the plasmid-containing strains to which was sub-

tracted the value obtained on the same strains but containing the vector pGE593.

Preparation of the TorR protein

TorR was purified to near homogeneity as described previously (Simon *et al*., 1994) except that Buffer A was replaced by Buffer B (40 mM Tris-HCl, pH 7.6) and the protein was eluted in the 500 mM KCl fraction. The protein concentration was estimated by the method of Bradford (1976).

Labelling of DNA fragment

For footprint assays, a DNA fragment corresponding to the *tna* promoter region was generated by PCR amplification with chromosomal DNA as template. The primers used for amplification were the same than those used for construction of the transcriptional fusion. The PCR product was labelled with [γ-^{32}P]-ATP (4000 Ci mmole^{-1}) using T4 polynucleotide kinase (Promega). The labelled fragment was separated from unincorporated nucleotides by using the Nucleotide Removal kit (QIAgen) and digested by *Bam*HI (Gibco-BRL).

DNase I footprinting

The footprint assays were performed as follows: the labelled DNA fragment was diluted to a concentration of about 1.5 nM in 50 µl of binding mix (10 mM Tris-HCl pH 7.5, 50 mM NaCl, 2.5 mM $MgCl_2$, 0.5 mM dithiothreitol, 4% glycerol and 40 ng µl^{-1} poly(dI-dC).poly(dI-dC)) to which different amount of TorR protein were added. After 30 min of incubation at room temperature, DNase I was added (0.66 U; Amersham Pharmacia Biotech) and the reaction was conducted for 1 min, then stopped by the addition of 140 µl of DNase Stop Solution (192 mM sodium acetate, 32 mM EDTA, 0.14% SDS and 64 µg ml^{-1} yeast RNA). After phenol/chloroform-extraction and DNA ethanol-precipitation, the pellets were resuspended in a loading solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) and loaded on a 8% polyacrylamide/6 M urea electrophoresis gel. The locations of the protected nucleotides were deduced by running a ladder with product of the G + A cleavage reaction (Maxam and Gilbert, 1980).

Construction of a tnaLAB *deletion mutant (LCB430)*

To delete the chromosomal *tnaLAB* operon in the MC4100 strain, we used the method described by Datsenko and Wanner (2000). First, the *tnaLAB* operon was replaced by a chloramphenicol-resistance gene generated by PCR. The primers were 60 nucleotides long and included 40-nucleotide homology extensions, corresponding to the 5′ region upstream of the start codon of the *tnaL* gene or to the 3′ extremity of the *tnaB* gene, and 20-nucleotide priming sequence that hybridized to the resistance gene flanking homologies of plasmid pKD3. The PCR mixture was treated with *Dpn*I and used to transform the MC4100 strain carrying the λ red helper plasmid pKD46. The resistance cassette was then eliminated by using plasmid pCP20 expressing the FLP recombinase which acts on the repeated sites flanking the resistance gene.

Bacterial survival assay

Overnight cultures of bacteria grown in L-broth were diluted (1/200) into fresh modified L-broth as previously specified. When indicated, the medium was supplemented with 40 mM of TMAO and/or 1 mM of tryptophan. The cells were grown during three days in anaerobiosis at 37°C. The pH was measured directly on the bacterial culture. The cultures were titred for viable cells at various times by plating on L-broth containing streptomycin. Values represent the averages of three experiments.

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Supplementary material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/mole/mole3428/mmi3428sm.htm

Each macroarray experiment is presented as a single file containing the following sections:

Activated genes.

Repressed genes.

ARRAY VISION data: raw data obtained with the ARRAY VISION software.

Blank cells: spots without DNA used to calculate an intensity threshold (first criteria).

Log of expression ratio: genes, matching the first criteria, used to calculate the log ratio threshold (second criteria).

References

Ansaldi, M., Bordi, C., Lepelletier, M., and Méjean, V. (1999) TorC apocytochrome negatively autoregulates the trimethylamine *N*-oxide (TMAO) reductase operon in *Escherichia coli*. *Mol Microbiol* **33:** 284–295.

Ansaldi, M., Simon, G., Lepelletier, M., and Méjean, V. (2000) The TorR high-affinity binding site plays a key role in both *torR* autoregulation and *torCAD* operon expression in *Escherichia coli*. *J Bacteriol* **182:** 961–966.

Ansaldi, M., Jourlin-Castelli, C., Lepelletier, M., Théraulaz, L., and Méjean, V. (2001) Rapid dephosphorylation of the TorR response regulator by the TorS unorthodox sensor in *Escherichia coli*. *J Bacteriol* **183:** 2691–2695.

Arfin, S.M., Long, A.D., Ito, E.T., Tolleri, L., Riehle, M.M., Paegle, E.S., and Hatfield, G.W. (2000) Global gene expression profiling in *Escherichia coli* K12. The effects of integration host factor. *J Biol Chem* **275:** 29672–29684.

Arnold, C.N., McElhanon, J., Lee, A., Leonhart, R., and Siegele, D.A. (2001) Global analysis of *Escherichia coli* gene expression during the acetate-induced acid tolerance response. *J Bacteriol* **183:** 2178–2186.

Barbosa, T.M., and Levy, S.B. (2000) Differential expression of over 60 chromosomal genes in *Escherichia coli* by constitutive expression of MarA. *J Bacteriol* **182:** 3467–3474.

Barrett, E.L., and Kwan, H.S. (1985) Bacterial reduction of trimethylamine oxide. *Annu Rev Microbiol* **39:** 131–149.

Blankenhorn, D., Phillips, J., and Slonczewski, J.L. (1999) Acid- and base-induced proteins during aerobic and anaerobic growth of *Escherichia coli* revealed by two-dimensional gel electrophoresis. *J Bacteriol* **181:** 2209–2216.

Botsford, J.L., and DeMoss, R.D. (1971) Catabolite repression of tryptophanase in *Escherichia coli*. *J Bacteriol* **105:** 303–312.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72:** 248–254.

Brocklehurst, K.R., and Morby, A.P. (2000) Metal-ion tolerance in *Escherichia coli*: analysis of transcriptional profiles by gene-array technology. *Microbiology* **146:** 2277–2282.

Castanié-Cornet, M.P., Penfound, T.A., Smith, D., Elliott, J.F., and Foster, J.W. (1999) Control of acid resistance in *Escherichia coli*. *J Bacteriol* **181:** 3525–3535.

Castanié-Cornet, M.P., and Foster, J.W. (2001) *Escherichia coli* acid resistance: cAMP receptor protein and a 20 bp *cis*-acting sequence control pH and stationary phase expression of the *gadA* and *gadBC* glutamate decarboxylase genes. *Microbiology* **147:** 709–715.

Datsenko, K.A., and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* **97:** 6640–6645.

De Biase, D., Tramonti, A., Bossa, F., and Visca, P. (1999) The response to stationary-phase stress conditions in *Escherichia coli*: role and regulation of the glutamic acid decarboxylase system. *Mol Microbiol* **32:** 1198–1211.

Deeley, M.C., and Yanofsky, C. (1981) Nucleotide sequence of the structural gene for tryptophanase of *Escherichia coli* K-12. *J Bacteriol* **147:** 787–796.

Edwards, R.M., and Yudkin, M.D. (1982) Location of the gene for the low-affinity tryptophan-specific permease of *Escherichia coli*. *Biochem J* **204:** 617–619.

Eraso, J.M., and Weinstock, G.M. (1992) Anaerobic control of colicin E1 production. *J Bacteriol* **174:** 5101–5109.

Gajiwala, K.S., and Burley, S.K. (2000) HDEA, a periplasmic protein that supports acid resistance in pathogenic enteric bacteria. *J Mol Biol* **295:** 605–612.

Gennis, R.B., and Stewart, V. (1996) Respiration. In *Escherichia coli and Salmonella*. Neidhardt, F.C. (ed). Washington, DC: American Society for Microbiology Press, pp. 217–261.

Gon, S., Jourlin-Castelli, C., Théraulaz, L., and Méjean, V. (2001) An unsuspected autoregulatory pathway involving apocytochrome TorC and sensor TorS in *Escherichia coli*. *Proc Natl Acad Sci USA* **98:** 11615–11620.

Grove, J., Tanapongpipat, S., Thomas, G., Griffiths, L., Crooke, H., and Cole, J. (1996) *Escherichia coli* K-12 genes essential for the synthesis of *c*-type cytochromes and a third nitrate reductase located in the periplasm. *Mol Microbiol* **19:** 467–481.

Hersh, B.M., Farooq, F.T., Barstad, D.N., Blankenhorn, D.L., and Slonczewski, J.L. (1996) A glutamate-dependent acid resistance gene in *Escherichia coli*. *J Bacteriol* **178:** 3978–3981.

Hommais, F., Krin, E., Laurent-Winter, C., Soutourina, O., Malpertuy, A., Le Caer, J.P., *et al.* (2001) Large-scale monitoring of pleiotropic regulation of gene expression by the prokaryotic nucleoid-associated protein, H-NS. *Mol Microbiol* **40:** 20–36.

Jourlin, C., Bengrine, A., Chippaux, M., and Méjean, V. (1996) An unorthodox sensor protein (TorS) mediates the induction of the *tor* structural genes in response to trimethylamine *N*-oxide in *Escherichia coli*. *Mol Microbiol* **20:** 1297–1306.

Jourlin, C., Ansaldi, M., and Méjean, V. (1997) Transphosphorylation of the TorR response regulator requires the three phosphorylation sites of the TorS unorthodox sensor in *Escherichia coli*. *J Mol Biol* **267:** 770–777.

Khodursky, A.B., Peter, B.J., Cozzarelli, N.R., Botstein, D., Brown, P.O., and Yanofsky, C. (2000) DNA microarray analysis of gene expression in response to physiological and genetic changes that affect tryptophan metabolism in *Escherichia coli*. *Proc Natl Acad Sci USA* **97:** 12170–12175.

Kirkpatrick, C., Maurer, L.M., Oyelakin, N.E., Yoncheva, Y.N., Maurer, R., and Slonczewski, J.L. (2001) Acetate and formate stress: opposite responses in the proteome of *Escherichia coli*. *J Bacteriol* **183:** 6466–6477.

Maxam, A.M., and Gilbert, W. (1980) Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol* **65:** 499–560.

McFall, E., and Newman, E.B. (1996) Amino acids as carbon sources. In *Escherichia coli and Salmonella*. Neidhardt, F.C. (ed). Washington, DC: American Society for Microbiology Press, pp. 358–379.

Méjean, V., Iobbi-Nivol, C., Lepelletier, M., Giordano, G., Chippaux, M., and Pascal, M.C. (1994) TMAO anaerobic respiration in *Escherichia coli*: involvement of the *tor* operon. *Mol Microbiol* **11:** 1169–1179.

Miller, J. (1972) *Experiments in Molecular Genetics.* Cold Spring Harbor, New York: Cold Spring Harbor Laboratory.

Oshima, T., Aiba, H., Masuda, Y., Kanaya, S., Sugiura, M., Wanner, B.L., *et al.* (2002) Transcriptome analysis of all two-component regulatory system mutants of *Escherichia coli*. *Mol Microbiol* **46:** 281–291.

Pascal, M.C., Lepelletier, M., Giordano, G., and Chippaux, M. (1991) A regulatory mutant of the trimethylamine *N*-oxide reductase of *Escherichia coli* K12. *FEMS Microbiol Lett* **62:** 297–300.

Pommier, J., Méjean, V., Giordano, G., and Iobbi-Nivol, C. (1998) TorD, a cytoplasmic chaperone that interacts with the unfolded trimethylamine *N*-oxide reductase enzyme (TorA) in *Escherichia coli*. *J Biol Chem* **273:** 16615–16620.

Pomposiello, P.J., Bennik, M.H., and Demple, B. (2001) Genome-wide transcriptional profiling of the *Escherichia*

coli responses to superoxide stress and sodium salicylate. *J Bacteriol* **183:** 3890–3902.

Simon, G., Méjean, V., Jourlin, C., Chippaux, M., and Pascal, M.C. (1994) The *torR* gene of *Escherichia coli* encodes a response regulator protein involved in the expression of the trimethylamine *N*-oxide reductase genes. *J Bacteriol* **176:** 5601–5606.

Simon, G., Jourlin, C., Ansaldi, M., Pascal, M.C., Chippaux, M., and Méjean, V. (1995) Binding of the TorR regulator to *cis*-acting direct repeats activates *tor* operon expression. *Mol Microbiol* **17:** 971–980.

Smith, D.K., Kassam, T., Singh, B., and Elliott, J.F. (1992) *Escherichia coli* has two homologous glutamate decarboxylase genes that map to distinct loci. *J Bacteriol* **174:** 5820–5826.

Stancik, L.M., Stancik, D.M., Schmidt, B., Barnhart, D.M., Yoncheva, Y.N., and Slonczewski, J.L. (2002) pH-dependent expression of periplasmic proteins and amino acid catabolism in *Escherichia coli*. *J Bacteriol* **184:** 4246–4258.

Stewart, V., and Yanofsky, C. (1985) Evidence for transcription antitermination control of tryptophanase operon expression in *Escherichia coli* K-12. *J Bacteriol* **164:** 731–740.

Stewart, V., and Yanofsky, C. (1986) Role of leader peptide synthesis in tryptophanase operon expression in *Escherichia coli* K-12. *J Bacteriol* **167:** 383–386.

Tanapongpipat, S., Reid, E., Cole, J.A., and Crooke, H. (1998) Transcriptional control and essential roles of the *Escherichia coli ccm* gene products in formate-dependent nitrite reduction and cytochrome *c* synthesis. *Biochem J* **334:** 355–365.

Tao, H., Bausch, C., Richmond, C., Blattner, F.R., and Conway, T. (1999) Functional genomics: expression analysis of *Escherichia coli* growing on minimal and rich media. *J Bacteriol* **181:** 6425–6440.

Tucker, D.L., Tucker, N., and Conway, T. (2002) Gene expression profiling of the pH response in *Escherichia coli*. *J Bacteriol* **184:** 6551–6558.

Wang, D., Ding, X., and Rather, P.N. (2001) Indole can act as an extracellular signal in *Escherichia coli*. *J Bacteriol* **183:** 4210–4216.

Yoshida, T., Ueguchi, C., and Mizuno, T. (1993) Physical map location of a set of *Escherichia coli* genes (*hde*) whose expression is affected by the nucleoid protein H-NS. *J Bacteriol* **175:** 7747–7748.

Zheng, M., Wang, X., Templeton, L.J., Smulski, D.R., LaRossa, R.A., and Storz, G. (2001) DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. *J Bacteriol* **183:** 4562–4570.

Zimmer, D.P., Soupene, E., Lee, H.L., Wendisch, V.F., Khodursky, A.B., Peter, B.J., *et al.* (2000) Nitrogen regulatory protein C-controlled genes of *Escherichia coli*: scavenging as a defense against nitrogen limitation. *Proc Natl Acad Sci USA* **97:** 14674–14679.