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The TorR High-Affinity Binding Site Plays a Key Role in Both *torR* Autoregulation and *torCAD* Operon Expression in *Escherichia coli*

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In the presence of trimethylamine *N*-oxide (TMAO), the TorS-TorR two-component regulatory system induces the *torCAD* operon, which encodes the TMAO respiratory system of *Escherichia coli*. The sensor protein TorS detects TMAO and transphosphorylates the response regulator TorR which, in turn, activates transcription of *torCAD*. The *torR* gene and the *torCAD* operon are divergently transcribed, and the short *torR-torC* intergenic region contains four direct repeats (the *tor* boxes) which proved to be TorR binding sites. The *tor* box 1-box 2 region covers the *torR* transcription start site and constitutes a TorR high-affinity binding site, whereas box 3 and box 4 correspond to low-affinity binding sites. By using *torR-lacZ* operon fusions in different genetic backgrounds, we showed that the *torR* gene is negatively autoregulated. Surprisingly, TorR autoregulation is TMAO independent and still occurs in a *torS* mutant. In addition, this negative regulation involves only the TorR high-affinity binding site. Together, these data suggest that phosphorylated as well as unphosphorylated TorR binds the box 1-box 2 region in vivo, thus preventing RNA polymerase from binding to the *torR* promoter whatever the growth conditions. By changing the spacing between box 2 and box 3, we demonstrated that the DNA motifs of the high- and low-affinity binding sites must be close to each other and located on the same side of the DNA helix to allow induction of the *torCAD* operon. Thus, prior TorR binding to the box 1-box 2 region seems to allow cooperative binding of phosphorylated TorR to box 3 and box 4.

Trimethylamine *N*-oxide (TMAO) is an organic compound widespread in nature, and very high levels of it accumulate in the tissues of fish, where it acts as a powerful osmoprotector (37). TMAO can also play the role of an alternative electron acceptor for bacterial anaerobic respiration (2, 3). In *Escherichia coli*, the genes encoding the TMAO reductase respiratory system are clustered in the *torCAD* operon (22). *torC* encodes a *c*-type cytochrome (TorC) anchored to the inner membrane, whereas *torA* encodes the periplasmic terminal enzyme (TorA). TorD, the product of the third gene of the *torCAD* operon, seems to be a TorA chaperone (28).

Expression of the *torCAD* operon is under the control of both anaerobiosis and TMAO or related compounds (35). The TMAO control is strict, as *torCAD* is rarely transcribed in the absence of TMAO (22). The anaerobic control is not as strong as that of TMAO, and expression of the *tor* operon decreases 5- to 10-fold under aerobic conditions (35). The *tor* anaerobic regulator, which is different from FNR or ArcA, is still unknown, and the TMAO control is mediated by the TorS-TorR two-component regulatory system (15, 35). We have shown that the sensor protein TorS seems to interact not only with TMAO but also with a periplasmic binding protein, TorT (16) and with the immature form of the TorC cytochrome (1). Whereas TorT is a positive regulator essential for *tor* operon induction, the apoform of TorC plays a negative autoregulatory role probably by inhibiting the TorS kinase activity. As TorS belongs to the family of the unorthodox sensors such as ArcB or BvgS (7, 24), the signal transduction from TorS to TorR involves a four-step phosphorelay (14). Once phosphorylated, TorR activates the *tor* operon transcription by binding to the *torCAD* promoter (34).

The short untranslated region between *torR* and *torC* contains four direct repeats of a decameric consensus motif (35) (see Fig. 2 and 3). These repeats have been called the *tor* boxes, and boxes 1, 2, and 4 correspond exactly to the same decameric sequence (CTGTTCATAT), whereas box 3 matches 7 of the 10 bases of the consensus (C**C**GTTCAT**C**C). By using plasmid-born *torC-lacZ* transcriptional fusions, we have observed that a double substitution within one of the four *tor* boxes leads to a strong decrease in fusion activity (34). This clearly indicates that the *tor* boxes are important *cis* elements involved in *tor* operon expression. We have further shown that TorR binds specifically to the four *tor* boxes. However, we noticed from gel retardation assays and footprinting experiments that the box 1-box 2 region constitutes a high-affinity binding site for unphosphorylated TorR, whereas box 3 and box 4 are lower-affinity binding sites (33, 34). In our working model, we proposed that, first, a dimer of unphosphorylated TorR protein binds the box 1-box 2 region. Under inducing conditions, TorR is transphosphorylated and oligomerizes to form at least a tetramer. Then, two subunits of the TorR tetramer interact with box 3 and box 4 leading to the induction of *tor* operon expression. Alternatively, TorR~P would first weakly bind as a dimer to box 3 and box 4, and this complex would then be stabilized by interaction with the TorR proteins previously bound to the box 1-box 2 site. In these models (34), we postulated that each TorR subunit binds one decameric direct repeat in a cooperative manner. This hypothesis is probably correct as TorR belongs to the OmpR family of response regulators (17, 21, 27, 35), and members of this family have

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been shown to bind cooperatively to multiple direct repeats (12, 29, 30, 38). Moreover, it has been reported that an OmpR binding site is comprised of two contiguous decameric repeats, more or less conserved, and that one OmpR molecule binds one DNA repeat (8, 9). The idea that emerges from several recent studies is that DNA binding of members of the OmpR family requires protein-protein interactions and occurs in a hierarchical manner so that high-affinity binding sites are filled first, and this binding facilitates interaction to weak binding sites by virtue of cooperativity (4, 10). Furthermore, phosphorylation of the response regulator seems to stimulate cooperative DNA binding (13).

As the TorR high-affinity binding site (box 1-box 2) covers the transcriptional start site of *torR* and overlaps its promoter −10 box (see Fig. 2), we decided to investigate whether or not the *torR* gene is autoregulated. We found that *torR* is negatively autoregulated and that full autoregulation requires an intact box 1-box 2 region. In contrast, the lower-affinity binding sites, box 3 and box 4, seem to play no role in this autogenous regulation. More surprisingly, *torR* autoregulation is unaffected in a *torS* mutant. Therefore, TorR seems to repress *torR* expression by binding the box 1-box 2 region even when it is unphosphorylated. Together, these results are consistent with our model in which the high-affinity binding site box 1-box 2 is bound by either the phosphorylated or the unphosphorylated form of TorR (34). In this model, the box 1-box 2 region plays the central role of a TorR oligomerization site, implying that the TorR binding site box 1-box 2 is properly positioned relative to box 3 and box 4 in order to allow the formation of a specific nucleoprotein complex able to activate *tor* operon transcription in inducing conditions. The effects produced by changing the spacing between box 2 and box 3 over the expression of a *torC-lacZ* fusion confirmed our proposal and showed that the motifs of the four boxes must be on the same side of the DNA helix. In addition, the activity of the *tor* operon promoter is at a maximum when the high- and low-affinity binding sites are close to each other.

MATERIALS AND METHODS

Strains, plasmids, growth conditions, and general methods. All strains used in this study are derivatives of strain LCB506 (MC4100 *pcnB*). Strain LCB434 is a *torS* null mutant (14), and strain LCB507 was constructed by P1 transduction into strain LCB506 of a *torR*::mini-Tn*10* allele (Cm^r), obtained by random mini-Tn*10* mutagenesis (1). The mini-Tn*10* insertion site was determined by a rapid inverse PCR method as previously described (1), and it corresponds to position 583 relative to the *torR* transcription start site. Bacteria were grown on L broth medium (23) in the presence of TMAO (10 mM) where indicated. To maintain selection for plasmids or to select for transductant strains, we used antibiotics as follows: ampicillin, 50 $\mu g \cdot ml^{-1}$; chloramphenicol, 25 $\mu g \cdot ml^{-1}$; tetracycline, 25 $\mu g \cdot ml^{-1}$, and spectinomycin, 25 $\mu g \cdot ml^{-1}$. Anaerobic cultures were grown overnight without shaking at 37°C in full-cap tubes. DNA preparations were carried out with the high pure DNA isolation kit from Boehringer Mannheim. PCR amplifications and DNA restrictions were carried out using standard procedures according to the supplier's instructions. Electrotransformations were performed by the rapid method of Enderle and Farwell (5).

Primer extension analysis. Strain MC4100 was grown anaerobically to late exponential phase with or without TMAO (10 mM). Total RNA was prepared by the hot-phenol method (23), the quality of the sample was checked electrophoretically, and quantification was done by spectroscopy. The two synthetic oligonucleotides complementary to sequences on the *torR* coding sequence (position 148 to 124 relative to the *torR* transcription start site) and the *torC* coding sequence (position 125 to 97 relative to the *torCAD* transcription start site) were end labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (U. S. Biochemicals) and were coprecipitated with 20 μg of RNA. The same amount of primer and RNA was used in each experiment. The primer extension reaction was performed with Moloney murine leukemia virus reverse transcriptase as previously described (22, 35). A sequencing ladder was produced with a DNA template corresponding to the 5′ *torC* region and the oligonucleotide used for the primer extension of the *torC* mRNA.

Construction of plasmids. The plasmid series pPR was created by PCR amplification of the *torR* promoter sequence from position −124 to position +15 for plasmids pPR1 to pPR4 and from position −53 to position +15 for pPR5, relative to the *torR* transcription start site, using chromosomal DNA as a template. We used mutagenic primers carrying two point mutations in box 1 (ATA TGAACAG→ATATG**CA**TAG), box 3 (GGATGAACGG→GGATG**CA**TGG), and box 4 (ATATGAACAG→ATATG**CA**TAG) for plasmids pPR2, pPR3, and pPR4, respectively. The PCR products, purified with Geneclean (BIO 101) and blunted using T4 DNA polymerase (Takara blunting kit), were then introduced into plasmid pGE593 (6) previously linearized with *Sma*I, thus placing the *lacZ* gene under the control of the *torR* promoter.

The plasmid series pPTor was created with a similar strategy by PCR amplification of the *torCAD* promoter sequence (from position −86 to position +276 relative to the *torCAD* transcription start site) and cloning into vector pGE593 (34). To create sequence insertions (pPTor28, -29, -32, and -33) or deletions (pPTor32 and -36) between box 2 and box 3, we performed PCR with insertion- or deletion-containing primers which start from the same 5′ extremity (see Fig. 3). Detailed information on the primer sequences is available from the authors on request.

All plasmids were checked by PCR with the upstream primer of the insert and a lacZ primer complementary to the *lacZ* sequence of pGE593 (1). The sequences of the PCR products were verified by direct sequencing with the lacZ primer.

β-Galactosidase assays. β-Galactosidase activity was measured on whole cells by the method of Miller (23); the measures were repeated at least three times to confirm reproducibility, and the standard deviation was no more than 15%.

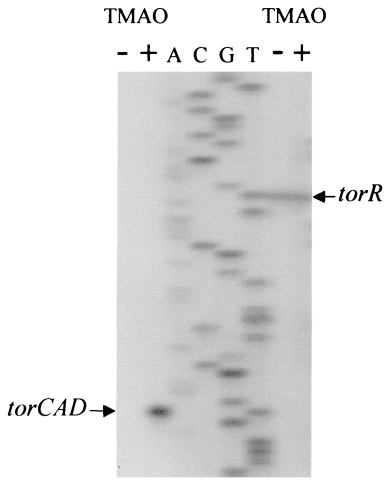
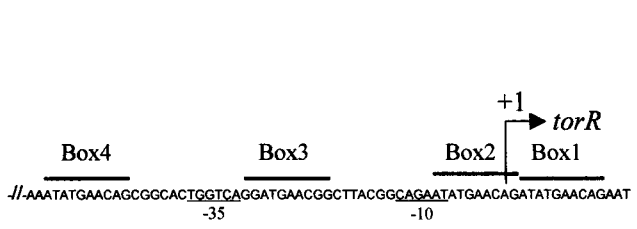


FIG. 1. Primer extension analysis of *torR* and *torCAD*. The labeled primers were annealed to RNA from MC4100 cells grown anaerobically in the absence (− lanes) or presence (+ lanes) of 10 mM TMAO and extended with reverse transcriptase. Lanes A, C, G, and T are a sequencing ladder of the *torC* DNA region made with the same primer as that used in the primer extension reaction of *torC*.

RESULTS AND DISCUSSION

Negative autoregulation of the *torR* gene. The *torR* gene and the *torCAD* operon are divergently transcribed, and their transcriptional start sites have been previously determined by primer extension (22, 35) (for the position of the transcription start sites see Fig. 2 and 3). From these experiments, we deduced that the *torR* and the *torCAD* promoters are back-to-back and so close that no intervening DNA sequence is found between the two promoter −35 boxes. As *torCAD* is expressed only in the presence of TMAO under anaerobic conditions, we wondered whether the *torR* gene was also regulated by TMAO. To answer this question, we carried out the same primer extension experiments as previously described, but RNA was prepared from cells grown either in the presence or absence of TMAO. As shown in Fig. 1, the level of *torR* transcription was almost the same in the presence or absence of TMAO. In contrast and as expected, no transcription was detected for the *torCAD* operon in the absence of TMAO, whereas *tor* operon transcription was observed in the presence of inducer.

To confirm that the *torR* promoter was constitutively ex-



Plasmids	β-Galactosidase activity					
	wt		*torR*		*torS*	
	TMAO +	TMAO -	TMAO +	TMAO -	TMAO +	TMAO -
pPR1	25	27	146	129	23	24
pPR2	104	99	172	150	89	102
pPR3	17	24	135	120	18	21
pPR4	23	22	203	178	30	29
pPR5	24	17	119	105	15	16

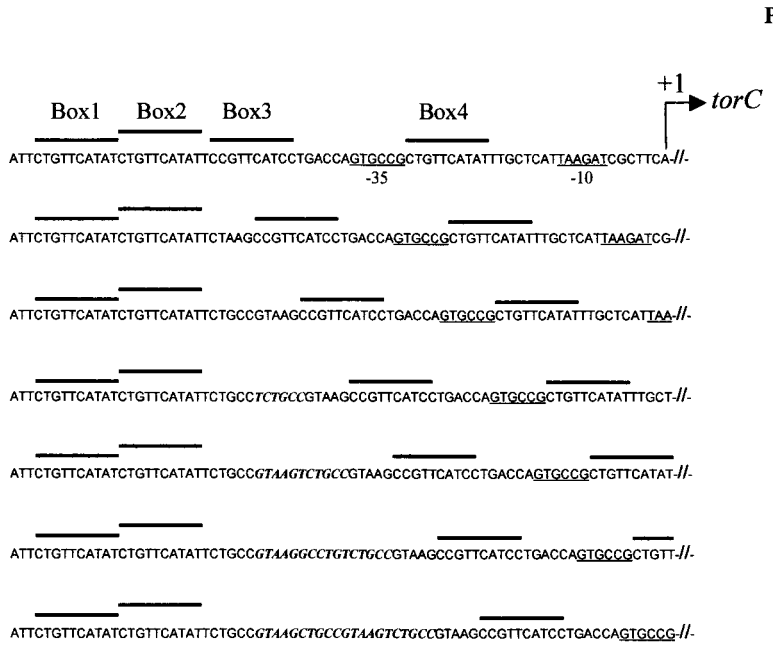
FIG. 2. Activity of the *torR* promoter mutated or not mutated on *tor* boxes in different genetic backgrounds. (Left) The *tor* boxes are overlined, and the transcription start site of *torR* is indicated by a +1 arrow. Except for pPR5, the 5′ part of the cloned *tor* sequence is not shown (as indicated by −//−). The 5′ end of the cloned *tor* fragment corresponds to position −124, relative to the *torR* transcription start site for pPR1 to pPR4 and to position −53 for pPR5; the 3′ extremity of the cloned fragment for all pPR plasmids corresponds to position +15. For plasmids pPR2, pPR3, and pPR4, only the point mutations are indicated. (Right) The LCB506 (*tor* wild-type [wt]), LCB507 (*torR*), and LCB434 (*torS*) strains containing the pPR plasmids were grown anaerobically in the presence (+) or absence (−) of TMAO. β-Galactosidase activity of the plasmid-borne *torR-lacZ* fusions is expressed in Miller units.

pressed whatever the growth conditions, we constructed a hybrid plasmid in which the *torR* promoter region was fused to the *lacZ* coding sequence of the operon fusion vector pGE593. In this plasmid (pPR1), the *tor* DNA fragment carries the four *tor* boxes and extends from position −124 to position +15 relative to the *torR* transcription start site (+1). To avoid any artifactual effect on fusion activity due to the high copy number of the plasmid, we introduced pPR1 and the other plasmids used in this study into *pcnB* strains. Because of the *pcnB* mutation, the copy number of the plasmid remains very low in this type of strain (18). In a *tor* wild-type context, the *torR-lacZ* fusion from pPR1 was expressed at almost the same low level in the presence or absence of TMAO under anaerobic conditions (Fig. 2). This result is consistent with the primer extension analysis described above and confirms that TMAO does not affect *torR* expression. In addition, expression of the *torR-lacZ* fusion was not significantly modified under aerobic growth conditions (data not shown). Therefore, in contrast to several response regulator genes (25, 36), the *torR* gene seems to be expressed at the same low level whatever the growth conditions.

As the TorR response regulator mediates TMAO induction of the *tor* operon promoter, we thought at first that the *torR* gene was constitutively expressed regardless of TorR. To test this hypothesis, we introduced pPR1 into a *torR* strain. Surprisingly, β-galactosidase activity was five- to sixfold higher than that measured in a *torR*$^+$ strain (Fig. 2). This result strongly suggests that the *torR* gene is negatively autoregulated, but an apparent paradox is that TorR autoregulation also takes place in the absence of TMAO (Fig. 1 and 2). An attractive possibility is that TorR can downregulate its own gene expression even when it is unphosphorylated. To clarify this last point, we decided to introduce pPR1 into a *torS* strain, since TorS transphosphorylates TorR in TMAO inducing conditions (14). As shown in Fig. 2, β-galactosidase activity measured in the *torS* strain is low whatever the growth conditions, and it is equivalent to that observed in a *tor* wild-type strain. TorS is thus not required for the TorR autoregulatory process. This result agrees with the fact that *torR* gene expression is unaffected by TMAO availability and supports the idea that TorR acts as a negative autoregulator in its phosphorylated, as well as unphosphorylated, form. Alternatively, a small amount of phosphorylated TorR might fulfil the autoregulatory function in the absence of TMAO, but this is unlikely because TorS is the only known sensor partner of TorR (1, 15).

TorR autoregulation requires only the high-affinity binding site. As the four *tor* boxes are necessary for *torCAD* operon induction and constitute TorR DNA-binding sites (34), we supposed that the same *cis*-acting elements were implicated in TorR negative autoregulation as well. To test this hypothesis, we separately changed the *tor* boxes 1, 3, and 4 by a double substitution as previously described (34). Indeed, substitutions at conserved positions 6 and 8 of the decameric consensus sequence (AAC to CAT [Fig. 2]) in any one of the four boxes strongly decreased the activity of a *torC-lacZ* fusion under inducing conditions. In the present study, we decided not to mutate *tor* box 2 because the *torR* transcription start site is located within this box, and mutations close to this site might artificially affect the level of expression of the *torR-lacZ* fusion. Figure 2 shows that the double substitution within *tor* box 1 (pPR2) increased the *torR-lacZ* fusion activity in the *tor* wild-type strain about fourfold. In contrast, expression of the *torR* promoter remained nearly unchanged when either box 3 (pPR3) or box 4 (pPR4) was mutated (Fig. 2). These results clearly indicate that *tor* box 3 and box 4 are not required for *torR* autoregulation, whereas box 1 is essential for this process. Considering that the box 1-box 2 region constitutes a single TorR binding site (33, 34), our results strongly suggest that this high-affinity binding site alone is responsible for *torR* negative autoregulation.

As a control, we also introduced the plasmids pPR2, pPR3, and pPR4 into the *torR* and *torS* strains (Fig. 2). As expected, the activity of the fusion was similar in both *torS* and *tor* wild-type strains for a given plasmid. This confirms that TorS plays no role in *torR* autoregulation and, consequently, expression of the *torR* promoter also increased in a *torS* strain when



Plasmids	bp	β-Galactosidase activity			
		wt		*torR*	
		TMAO		TMAO	
		+	-	+	-
pPTor34	-10	535	9	<5	<5
pPTor36	-5	7	8	ND	ND
pPTor16	0	378	7	<5	<5
pPTor28	+6	8	10	ND	ND
pPTor29	+11	70	6	<5	<5
pPTor32	+16	6	6	ND	ND
pPTor33	+21	24	8	<5	<5

FIG. 3. Activity of the *tor* operon promoter with base pair deletions or insertions between box 2 and box 3. (Left) The *tor* boxes are overlined, the sequence insertions are in italics, and the transcription start site of *torC* is indicated by a +1 arrow. The 3′ part of the cloned *tor* sequence is not shown (as indicated by −//−). The 3′ end of the cloned *tor* fragment corresponds to position +275. (Right) The LCB506 (*tor* wild-type [wt]) and LCB507 (*torR*) strains containing the pPTor plasmids were grown anaerobically in the presence (+) or absence (−) of TMAO. β-Galactosidase activity of the plasmid-borne *torC-lacZ* fusions is expressed in Miller units. bp, the number of base pairs inserted (+) or deleted (−) between box 2 and box 3; ND, not determined.

box 1 was mutated. In a *torR* context, activity originating from plasmids pPR2, pPR3, or pPR4 reached levels similar to that measured from pPR1, and this activity was always higher in the *torR* strain than in the other two strains. Together, these results highlight the negative action of TorR on *torR* gene expression. The fact that activity was higher in the *torR* strain even for the box 1-mutated *torR* promoter (pPR2) (Fig. 2) indicates that the effect of the mutations in box 1 is not as strong as that of *torR* inactivation. This is consistent with previous findings that showed that a double mutation in either box 1 or box 2 did not entirely prevent TorR binding to the box 1-box 2 region (33, 34). Thus, TorR might still slightly downregulate its own expression by binding loosely to the mutated box 1-box 2 region.

To confirm that repression of the *torR* promoter only requires TorR binding to the box 1-box 2 region, we cloned a small DNA fragment carrying just the *torR* promoter from position −53 to +15 relative to the *torR* transcription start site into pGE593. As expected, expression from this fusion (pPR5) (Fig. 2) remained almost unchanged in a wild-type or *torS* context, whereas it increased about sixfold in a *torR* strain. Together, these results clearly show that TorR negative autoregulation does not require auxiliary sites in addition to the box 1-box 2 region. As the box 1-box 2 TorR binding region overlaps the RNA polymerase binding region of the *torR* promoter, the interaction of TorR with this region might prevent RNA polymerase binding to the *torR* promoter by a simple steric hindrance mechanism. This repression mechanism is probable because most repressors act by limiting the access of the RNA polymerase to the promoter, and steric hindrance is one of the classical mechanisms used by repressors to achieve their function (26, 32).

TorR high- and low-affinity binding sites must be properly positioned to each other to allow *tor* operon induction. It is striking that the DNA motifs of the *tor* boxes are found on the same side of the DNA helix (Fig. 2). This observation is consistent with our previous proposal in which TorR binds first as a dimer to the box 1-box 2 region in its unphosphorylated form and then interacts, under inducing conditions, with the weak binding sites, boxes 3 and 4, owing to cooperative interactions stimulated by phosphorylation (34). Formation of such an active nucleoprotein complex requires that the four TorR subunits are located close to each other on the same side of the DNA helix. A similar model has been recently proposed for regulation of *ompF* by OmpR (13), and activation of the *pstS* gene involves a DNA-PhoB complex that resembles the proposed DNA-TorR complex (19). However, an 11-bp intervening sequence is found between the high- and low-affinity binding sites in the case of the *tor* operon promoter. To examine the role of this intervening sequence over *tor* operon expression and to check that the TorR binding sites must be present on the same side of the DNA helix, we decided to change the distance between box 2 and box 3.

Figure 3 summarizes the effects of small insertions or deletions within the box 2-box 3 intervening region over a plasmid-borne *torC-lacZ* fusion. When the intervening region was changed so that the box 1-box 2 and box 3-box 4 DNA motifs were positioned on opposite sides of the DNA helix (pPTor28, pPTor32, and pPTor36), expression of the *torC-lacZ* fusion was very low whatever the growth conditions. In contrast, when the box 1-box 2 and box 3-box 4 DNA motifs were positioned on the same side of the DNA helix (pPTor29, pPTor33, and pPTor34), the fusion activity increased in the presence of TMAO. These results show that the DNA motifs of the high- and low-affinity binding sites must be positioned on the same face of the DNA helix to allow *tor* operon induction. Therefore, the TorR subunits activate *tor* operon expression by binding to the same side of the DNA helix.

Although a proper phasing between the *tor* boxes seems to

be essential for *tor* operon expression, the distance between the high- and low-affinity binding sites appears to play an important role in the strength of the *tor* operon promoter. Indeed, insertion of one (pPTor29) or two (pPTor33) additional helical turns between box 2 and box 3 led to a strong decrease in *torC-lacZ* fusion activity under inducing conditions (Fig. 3). Furthermore, deletion of the box 2-box 3 intervening region (pPTor34) resulted in an even higher fusion activity than that of the wild-type promoter fusion. From this experiment, we conclude that the closer the high- and low-affinity binding sites are, the higher *tor* operon expression is. Finally, we introduced the same plasmids into a *torR* strain and, as expected, no activity above the background level was measured (Fig. 3).

Concluding remarks. In the present study, we show that the TorR response regulator is not only an activator of the *tor* structural operon but also a repressor of its own gene expression (20, 32). The *torR* gene and the *tor* operon are divergently transcribed, and our data indicate that the box 1-box 2 TorR high-affinity binding site, located within the *torR-torC* intergenic region, is absolutely required for both repression of *torR* and activation of *torCAD*. The fact that binding of TorR to the weaker binding sites of box 3 and box 4, which are essential for *tor* operon expression, does not enhance *torR* repression strongly suggests that in contrast to many repressors (26, 32) TorR does not need to bind auxiliary binding sites to achieve full repression. In addition, TorR seems to bind the box 1-box 2 region in vivo in its unphosphorylated form, since *torR* negative autoregulation depends on neither the TorS sensor partner nor the presence of TMAO (Fig. 1 and 2). Accordingly, *torR* negative autoregulation is likely to maintain TorR concentration at a low level whatever the growth conditions.

Although the box 2-box 3 intervening sequence is essential for *torR* gene expression, as it is located within the *torR* promoter and carries part of the *torR* promoter −10 box, this DNA region proved to be dispensable not only for TMAO induction of the *tor* operon (Fig. 3) but also for the anaerobic control of the same operon (data not shown). However, *tor* operon induction requires strict spacing between the high- and low-affinity binding sites, so that the repeat sequences of the four *tor* boxes are oriented to the same face of the DNA helix. This further supports our previous model concerning the formation of an active nucleoprotein complex in which a TorR~P tetramer binds the four *tor* boxes simultaneously and probably bends the *tor* regulatory region (34).

To extend our model, we propose now that RNA polymerase binds the *torR* promoter on the same side as TorR. Therefore, repression efficiency might depend mainly on the competition between TorR and the RNA polymerase for their overlapping binding sites. If this is true, then transcription of the *torR* gene should occur only in the absence of TorR binding, and as expression of the *torCAD* operon is strictly TorR-dependent, transcription initiations of *torR* and *torCAD* might be mutually exclusive. Additional experiments are required to better understand this complex regulatory process. It also remains to be answered how TorR activates transcription of the *tor* operon and whether or not the RNA polymerase that binds the *tor* operon promoter is positioned on the same side as TorR (11, 31).

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