The GntR-Like Regulator TauR Activates Expression of Taurine Utilization Genes in *Rhodobacter capsulatus*[▽]

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***Rhodobacter capsulatus* can efficiently grow with taurine as the sole sulfur source. The products of the *tpa-tauR-xsc* gene region are essential for this activity. TauR, a MocR-like member of the GntR superfamily of transcriptional regulators, activates *tpa* transcription, as shown by analysis of wild-type and *tauR* mutant strains carrying a *tpa-lacZ* reporter fusion. Activation of the *tpa* promoter requires taurine but is not inhibited by sulfate, which is the preferred sulfur source. TauR directly binds to the *tpa* promoter, as demonstrated by DNA mobility shift assays. As expected for a transcriptional activator, the TauR binding site is located upstream of the transcription start site, which has been determined by primer extension. Site-directed promoter mutations reveal that TauR binds to direct repeats, an unusual property that has to date been shown for only one other member of the MocR subfamily, namely, GabR from *Bacillus subtilis*. In contrast, all other members of the GntR family analyzed so far bind to inverted repeats.**

Sulfate is the preferred sulfur source of most bacteria, but in most natural environments, sulfate availability is limited. Sulfonates and sulfate esters are much more abundant than sulfate, and consequently, many bacteria have adapted mechanisms to use these compounds as alternative sulfur sources (10). Upon sulfate limitation, *Escherichia coli* and many other bacteria synthesize a set of sulfate starvation-induced proteins (references 10, 24, and 25 and references therein). Among these are ABC-type transporters involved in uptake of taurine (2-aminoethanesulfonate).

Phototrophic purple bacteria (*Rhodospirillaceae*), and especially *Rhodobacter* species, have long been used as model organisms to study anoxygenic photosynthesis, carbon assimilation, hydrogen metabolism, and nitrogen fixation (references 6–8 and 15 and references therein). In the absence of sulfate, *Rhodobacter capsulatus* can efficiently grow with taurine under photoheterotrophic conditions (16).

Two divergently transcribed gene clusters, *tauABC* and *tpa-tauR-xsc* (formerly *orf459-orf484-orf590*), are involved in taurine utilization by *Rhodobacter capsulatus* (16) (Fig. 1). The *tauABC* genes are predicted to encode an ABC transport system mediating taurine uptake. The *tpa* and *xsc* gene products exhibit strong similarity to taurine-pyruvate aminotransferase (Tpa) from *Bilophila wadsworthia* (catalyzing the initial transamination of taurine to 2-sulfoacetaldehyde during anaerobic taurine degradation) (13) and sulfoacetaldehyde acetyltransferase (Xsc) from *Alcaligenes defragrans* (converting 2-sulfoacetaldehyde into sulfite and acetyl phosphate) (21). Genes similar to *R. capsulatus tauR* were previously identified in close proximity to *xsc* genes in the genomes of *Paracoccus denitrificans* and other proteobacteria by database searches (4, 21), but none of these genes has been experimentally characterized so

far. As shown in this study, TauR is a transcriptional activator essential for *tpa* expression.

R. capsulatus TauR belongs to the GntR superfamily of bacterial transcription regulators that bind DNA by a helix-turn-helix motif. The N-terminal DNA-binding domain is well conserved for all members of the GntR family, whereas the C-terminal effector-binding and oligomerization domain is more variable and therefore was used to define six GntR subfamilies, namely, the FadR, HutC, MocR, YtrA, AraR, and PlmA families (14, 19). Members of the GntR family control diverse processes, such as fatty acid metabolism in *E. coli* (FadR), histidine utilization in *Pseudomonas putida* (HutC), rhizopine catabolism in *Rhizobium meliloti* (MocR), acetoin utilization in *Bacillus subtilis* (YtrA), arabinose metabolism in *B. subtilis* (AraR), and plasmid copy number control in *Anabaena* sp. strain PCC 7120 (PlmA). *R. capsulatus* TauR shows the highest similarity to the MocR subfamily, named after *S. meliloti* MocR (20).

In contrast to well-characterized members of the FadR, HutC, and YtrA subfamilies, which bind to inverted repeats in the promoter regions of their target genes, MocR-like proteins had previously been predicted to bind to direct repeats (19). To date, this has been shown for only one MocR-like protein, namely, GabR from *B. subtilis* (2, 3). GabR acts as both a negative autoregulator and an activator of genes involved in the utilization of γ -aminobutyrate (GABA) as the sole nitrogen source.

In this study, we analyzed the role of *R. capsulatus* TauR by genetic and biochemical means. TauR was shown to be essential for taurine-dependent *tpa* expression. Site-directed mutagenesis of the *tpa* promoter and DNA mobility shift assays suggest binding of TauR to direct repeats.

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MATERIALS AND METHODS

Strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. Methods for conjugational plasmid transfer between *E. coli* and *R. capsulatus*, procedures for selection of mutants,

FIG. 1. Organization of the *R. capsulatus* *tpa-tauR-xsc* gene region. Mutant strains defective for *tpa*, *tauR*, and *xsc* contain Gm resistance cassettes, with the directions of transcription of the Gm resistance gene symbolized by arrows. Hybrid plasmid pBSRUB60 carrying a transcriptional fusion of *lacZ* to the *tpa* promoter is based on the mobilizable broad-host-range plasmid pBBR1MCS. Neither the Gm resistance cassette nor the *lacZ* gene is drawn to scale.

and growth conditions have been described earlier (reference 16 and references therein).

RNA isolation and primer extension. *R. capsulatus* cultures were grown in RCV minimal medium with taurine as a sulfur source until late logarithmic phase. RNAs of these cultures were isolated using the Micro-to-Midi Total RNA Purification System (Invitrogen, Karlsruhe, Germany) following the instructions of the manufacturer. Primer extension was carried out as described previously (1) using oligonucleotide LP-*tpa*-2, complementary to the 5' end of *tpa* mRNA (Table 2), to map the transcription start site of *tpa* (Fig. 2).

Construction of the P_{tpa} -*lacZ* reporter plasmid pBSRUB60 and β -galactosidase assays. A 461-bp DNA fragment (Fig. 3, fragment A) encompassing the *tpa* promoter (P_{tpa}) was PCR amplified with the primer pair UP-*tpa*-1/LP-*tpa*-2 (Table 2), using *R. capsulatus* chromosomal DNA as a template. The PCR product was blunt-end cloned into the SmaI site of vector plasmid pBluescript KS, leading to plasmid pBSRUB48, in which P_{tpa} was flanked by BamHI and HindIII restriction sites. Subsequently, the BamHI-HindIII fragment from pBSRUB48 carrying P_{tpa} was cloned into the mobilizable broad-host-range plasmid pBBR1MCS. Finally, the promoterless *E. coli lacZ* gene was inserted at the HindIII site, resulting in reporter plasmid pBSRUB60 (*tpa-lacZ*). *R. capsulatus* reporter strains carrying pBSRUB60 (*tpa-lacZ*) were grown in RCV minimal medium with taurine or sulfate as a sulfur source before β -galactosidase activities were determined as described earlier (16).

Overexpression of *R. capsulatus* TauR protein in *E. coli*. The *tauR* coding region was PCR amplified with the primer pair UP-*tauR*/LP-*tauR* (encompassing recognition sites for SacII and SalI, respectively) (Table 2) using *R. capsulatus* chromosomal DNA as a template. Subsequently, the 1.5-kb SacII-SalI DNA fragment with *tauR* was cloned into the Strep-tag expression vector pASK-IBA3 (Table 1), resulting in hybrid plasmid pBSRUB29, carrying an in-frame *tauR*-Strep-TagII fusion (*tauR*_{Strep}). Plasmid pBSRUB29 was transformed into *E. coli* strain BL21(DE3), which served as a host for overexpression of the tagged *R. capsulatus* TauR protein (TauR_{Strep}). Purification of the recombinant protein was carried out as described previously (26).

Nonradioactive DNA mobility shift assays. A 461-bp DNA fragment encompassing P_{tpa} was obtained by PCR amplification with the primer pair UP-*tpa*-1/LP-*tpa*-2 using plasmid pBSRUB48 as a template (Table 2). P_{tpa} subfragments were generated by cutting the 461-bp amplification product with appropriate restriction enzymes (Fig. 3). Preincubation of promoter fragments with the TauR_{Strep} protein and agarose gel electrophoresis of DNA-protein complexes were carried out as described previously (18).

Site-directed mutagenesis and radioactive DNA mobility shift assays. Point mutations within the *tpa* promoter region (designated mut-1 to mut-4) (Fig. 4) were generated by overlap extension PCR (23) with appropriate primers (Table 2) and pBSRUB48 as a template. The PCR products were blunt-end cloned into the SmaI site of pBluescript KS, leading to hybrid plasmids pBSRUB94 (mut-1), pBSRUB95 (mut-2), pBSRUB96 (mut-3), pBSRUB97 (mut-4), and pBSRUB110 (mut-3/4). These plasmids served as templates for PCR amplification of 269-bp mutant *tpa* promoter fragments using the primer pair PJW72-U/PJW73-L (Table

FIG. 2. Transcription start site mapping of the *tpa* promoter. Primer extension was carried out with total RNA from *R. capsulatus* cells grown with taurine as the sole sulfur source. Primer LP-*tpa*-2 (binding to the 5' region of *tpa*) (Table 2) was used for reverse transcription. The resulting primer extension product is shown in lane PE. The corresponding sequencing reactions (A, C, G, and T) with plasmid pBSRUB48 served as length standards. The putative (−10) promoter region, the transcription start site (+1), the ribosomal binding site (RBS), and the translation initiation codon for the *tpa* gene product (Tpa) are indicated.

2). Purification of the amplification products, 32 P end labeling, preincubation of labeled promoter DNA with the TauR_{Strep} protein, and polyacrylamide gel electrophoresis of DNA-protein complexes were carried out as described earlier (27). If required, 1 μ M taurine or 100 μ M pyridoxal 5'-phosphate was added to the preincubation mixture. Quantification of DNA mobility shift assays was done using the AIDA image analyzer software, version 4.19 (Raytest, Straubenhardt, Germany).

RESULTS AND DISCUSSION

Mapping of the *tpa* transcription start site. Genetic analyses have indicated that the *tpa*, *tauR*, and *xsc* genes comprise a single transcription unit (reference 16 and this study) (Fig. 1). To assign the transcription start site of the *tpa-tauR-xsc* operon, primer extension experiments were performed with total RNA isolated from an *R. capsulatus* wild-type culture grown with taurine as the sole sulfur source (see Materials and Methods) (Fig. 2). The 5' end of the mRNA mapped between the TauR binding site (see below) (Fig. 3) and the ATG start codon for the *tpa* gene.

TauR activates *tpa* expression. To analyze the expression of the *tpa-tauR-xsc* operon, plasmid pBSRUB60 carrying a transcriptional *tpa-lacZ* fusion was introduced into wild-type *R. capsulatus* and *tpa*, *tauR*, and *xsc* mutant strains (see Materials and Methods) (Table 3). These mutant strains contain a gentamicin (Gm) resistance cassette, which constitutively drives transcription of downstream genes (reference 27 and references therein). The resulting reporter strains were grown in RCV minimal medium with taurine or sulfate as a sulfur source prior to determination of *lacZ*-mediated β -galactosidase activity, as described earlier (16). The results shown in Table 3 can be summarized as follows. (i) Expression of the *tpa-lacZ* fusion was taurine inducible, as expected from earlier studies (16). In contrast to *R. capsulatus*, the *tpa* and *xsc* genes

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics ^a	Reference or source
<i>E. coli</i>		
BL21(DE3)	Host for overexpression of TauR _{Strep}	Novagen, Darmstadt, Germany
DH5α	Host for plasmid construction	9
S17-1	Donor providing transfer functions	22
<i>R. capsulatus</i>		
B10S	Spontaneous Sm-resistant mutant of <i>R. capsulatus</i> B10	11
FFRUB33	Δ(<i>tpa-tauR-xsc</i>):[<Gm]	16
FFRUB47	<i>tpa</i> ::[<Gm]	16
FFRUB48	<i>tpa</i> ::[Gm>]	16
FFRUB53	<i>tauR</i> ::[Gm>]	16
FFRUB54	<i>tauR</i> ::[<Gm]	16
FFRUB56	<i>xsc</i> ::[Gm>]	16
Plasmids		
pASK-IBA3	Strep tag expression vector; <i>tet</i> p/o; <i>tetR</i> Ap	IBA, Göttingen, Germany
pBBR1MCS	Mobilizable broad-host-range vector	12
pBluescript KS	High-copy-number vector, Ap	Stratagene, Amsterdam, The Netherlands
pBSRUB29	pASK-IBA3 derivative carrying <i>tauR</i> _{Strep}	This study
pBSRUB48	pBluescript KS derivative carrying a 461-bp PCR product with P _{tpa}	This study
pBSRUB60	pBBR1MCS derivative carrying a P _{tpa} - <i>lacZ</i> fusion; Tc	This study
pBSRUB94	pBluescript KS derivative carrying P _{tpa} variant mut-1	This study
pBSRUB95	pBluescript KS derivative carrying P _{tpa} variant mut-2	This study
pBSRUB96	pBluescript KS derivative carrying P _{tpa} variant mut-3	This study
pBSRUB97	pBluescript KS derivative carrying P _{tpa} variant mut-4	This study
pBSRUB110	pBluescript KS derivative carrying P _{tpa} variant mut-3/4	This study

^a Ap, ampicillin; Km, kanamycin; Tc, tetracycline; *tet* p/o, tetracycline promoter/operator; *tetR*, tetracycline repressor gene; Sm, streptomycin Gm, gentamicin. Arrowheads indicate cassette orientation.

FIG. 3. DNA mobility shift assays narrowing down the TauR binding site within the *tpa* promoter region. The transcription start site of the *tpa* gene (labeled +1) is indicated above the physical map. DNA fragment A (461 bp) encompassing the *tauA-tpa* intergenic region was generated by PCR amplification using the primer pair UP-*tpa*-1/LP-*tpa*-2 (Table 2). In addition, subfragments were generated by cutting fragment A with the indicated restriction enzymes. The DNA fragments were either preincubated with purified TauR_{Strep} protein (lanes 2, 4, 6, 8, and 10) or not (lanes 1, 3, 5, 7, and 9) prior to agarose gel electrophoresis. DNA fragments retarded by TauR are marked (A to E). A 50-bp DNA ladder (Fermentas, St. Leon-Rot, Germany) was used as a length standard (lane M).

in *Rhodobacter sphaeroides* belong to different operons. While Xsc and other enzymes involved in the utilization of taurine as a sole carbon or nitrogen source by *R. sphaeroides* are synthesized upon the addition of taurine, expression of the *tpa* gene is not induced by taurine (5). Thus, even closely related species, such as *R. capsulatus* and *R. sphaeroides*, have adopted various strategies for taurine utilization. (ii) Transcription from the *tpa* promoter was not inhibited by sulfate. In contrast, expression of the *tauABC* genes coding for a putative ABC-type taurine transporter was clearly repressed by sulfate (16). Nevertheless, small amounts of the TauABC transporter should be synthesized in the presence of sulfate in order to allow uptake of small amounts of taurine sufficient for TauR-dependent *tpa* activation. Alternatively, some taurine might enter the cells independently of the sulfate-inhibited TauABC transporter. However, utilization of taurine as a sulfur source seems to be primarily controlled by sulfate repression of taurine uptake, while utilization of (intracellular) taurine is not inhibited by sulfate. This might reflect the adaptation of *R. capsulatus* to natural environments, which are characterized by much lower and more varied concentrations of sulfate and taurine than the experimental conditions used in this study. (iii) Taurine-dependent induction of *tpa* expression strictly required TauR. (iv) In contrast to TauR, neither Tpa nor Xsc was essential for *tpa* expression. It remains unclear, however, why the *xsc* mutation led to elevated levels of *tpa-lacZ* expression in the presence of taurine plus sulfate. (v) A polar *tpa* mutation (with the Gm gene reading in the opposite direction

TABLE 2. Synthetic oligonucleotides used in this study

Oligonucleotide	DNA sequence (5'→3') ^a	Relevant characteristics
UP-tauR	CCCGCGGAAGCTGCGAGGTCAAGATGGCC	Amplification of <i>tauR</i> coding region; 1,485 bp
LP-tauR	GCAGGTCGACACGACGGGCGCGGGCGATG	
UP-tpa-1	GCGCGGCACGTTTCAGGGCTTTGGTCAT	<i>tpa</i> promoter amplification; 461 bp; primer extension
LP-tpa-2	CGGATCGCTGGTTTCATAGGGCTTGTGCT	
UP-mut-1	GGCGGGGGAAGAATATTCCTCCGTCAGGGTGG	Site-directed mutagenesis of the <i>tpa</i> promoter
LP-mut-1	CCACCCTGACGGGAATATTTCTCCCCCGCC	
UP-mut-2	CCCGTCAGGGTGCACAAAACGGCGCTCTGG	Site-directed mutagenesis of the <i>tpa</i> promoter
LP-mut-2	CCAGAGCGCCGTTTTGTGACCCCTGACGGG	
UP-mut-3	CGGCGCTCTGCAGTTAAGGCTTCGGGG	Site-directed mutagenesis of the <i>tpa</i> promoter
LP-mut-3	CCCCGAAGCCTTAAGTGCAGAGCGCCG	
UP-mut-4	GGCTTCGGGGAAACTGCAGCTAATCGAAGCGGGG	Site-directed mutagenesis of the <i>tpa</i> promoter
LP-mut-4	CCCCGCTTCGATTAGCTGCAGTTTCCCCGAAGCC	
PJW72-U	TCAGAGGTGGCGGAATGTT	<i>tpa</i> promoter amplification; 269 bp
PJW73-L	CCCCCGCTTCGATTAGGTCCAGT	

^a The underlined sequences are recognition sites for PstI, SacII, SalI, and SspI.

from *tpa*; FFRUB47) prevented *tpa-lacZ* expression, suggesting that *tauR* expression derives from the *tpa* promoter. Thus, this finding implies cotranscription of *tpa* and *tauR*. (vi) Expression of *tauR* by the constitutive Gm gene promoter (FFRUB48) led to elevated levels of *tpa-lacZ* expression, indicating that *tauR* was overexpressed in the mutant. In this mutant, clear *tpa* expression occurred in the presence of sulfate as the sole sulfur source, suggesting that TauR mediates *tpa* activation to some extent independently of taurine availability. In the wild type, taurine-independent low-level expression of

the *tpa-tauR-xsc* operon may account for basal levels of TauR required for positive autoregulation.

TauR binds to the *tpa* promoter. Since *R. capsulatus* TauR was essential for *tpa-lacZ* expression (Table 3), it seemed likely that TauR would bind to the *tpa* promoter. Interaction of TauR with the *tpa* promoter was analyzed by DNA mobility shift assays (see Materials and Methods). For this purpose, the TauR protein was overexpressed and purified as a C-terminally Strep-tagged recombinant protein from *E. coli* (see Materials and Methods). TauR_{Strep} was preincubated either with a

FIG. 4. Site-directed mutagenesis of the *tpa* promoter. The heavy line below the physical map depicts a 269-bp PCR fragment (amplified with the primer pair PJW72-U/PJW73-L [Table 2]) used for DNA mobility shift assays (Fig. 5). Site-directed mutations (mut-1, mut-2, mut-3, and mut-4) within direct repeats (DR-1a, DR-1b, DR-2a, and DR-2b) were created by overlap extension PCR. Direct repeats DR-2a and DR-2b are centered at positions -72.5 and -49.5, respectively, relative to the transcription start site (+1).

TABLE 3. Taurine-dependent expression of *tpa-lacZ* in *R. capsulatus*

Strain ^a	Relevant characteristics	β -Galactosidase activity ^b		
		Sulfate	Taurine	Taurine + sulfate
B10S	Wild type	6 $\pm$ 1	128 $\pm$ 3	109 $\pm$ 7
FFRUB33	$\Delta(tpa-tauR-xsc)$	2 $\pm$ 1	0 $\pm$ 0	2 $\pm$ 1
FFRUB47	<i>tpa</i> ; polar	1 $\pm$ 1	1 $\pm$ 1	2 $\pm$ 2
FFRUB48	<i>tpa</i>	168 $\pm$ 13	866 $\pm$ 110	982 $\pm$ 23
FFRUB54	<i>tauR</i> ; polar	1 $\pm$ 0	1 $\pm$ 1	3 $\pm$ 3
FFRUB53	<i>tauR</i>	3 $\pm$ 2	2 $\pm$ 2	2 $\pm$ 2
FFRUB56	<i>xsc</i>	3 $\pm$ 1	74 $\pm$ 11	369 $\pm$ 59

^a All *R. capsulatus* strains contained plasmid pBSRUB60 (*tpa-lacZ*) based on the broad-host-range vector pBBR1MCS.

^b *R. capsulatus* strains were grown in minimal medium with the indicated sulfur sources. β -Galactosidase activity is given in Miller units (17). The results represent the means and standard deviations of three independent measurements.

461-bp PCR fragment encompassing the *tpa* promoter or with subfragments created by digestion of the PCR product with different restriction enzymes. The protein-DNA complexes were analyzed by agarose gel electrophoresis, followed by ethidium bromide staining (Fig. 3). Since only one of two re-

striction fragments in the respective assays was retarded, the other fragment served as an internal control for TauR-specific binding. Comparison of retarded DNA fragments suggested that the TauR binding site is confined to an 88-bp BglI-TacI fragment within the *tpa* promoter region. As one would expect for the binding site of a transcriptional activator, the TauR binding site is located upstream of the transcription start site (Fig. 3).

Since *tpa-lacZ* expression was taurine inducible in vivo (Table 3), we asked whether taurine might influence in vitro binding of TauR to the *tpa* promoter. The *tpa* promoter region was PCR amplified using the primer pair PJW72-U/PJW73-L (Table 2) prior to radioactive 5' end labeling of the 269-bp PCR product (Fig. 4). TauR_{Strep} was preincubated with the radioactively labeled *tpa* promoter fragment in the presence or absence of taurine. Analysis of protein-DNA complexes by polyacrylamide gel electrophoresis revealed specific binding of TauR to the *tpa* promoter in the absence of taurine (Fig. 5A and B). TauR binding was not further enhanced by taurine. Thus, taurine-independent binding of the regulator to DNA and taurine-dependent gene activation are two separate events (see "Conclusions" below).

Members of the MocR subfamily of transcriptional regula-

FIG. 5. Influences of taurine and PLP on binding of TauR to the *tpa* promoter and specificity controls. (A) A 269-bp DNA fragment (marked in Fig. 4) carrying the TauR binding site was ³²P end labeled prior to incubation with increasing amounts of TauR_{Strep} (0, 0.02, 0.04, 0.07, 0.15, 0.22, 0.29, 0.44, 0.58, and 1.17 μ M) in the absence or presence of taurine and/or PLP (see Materials and Methods). All binding assays were performed in the presence of poly(dI-dC) as the competitor DNA. (B) The decrease of band intensities for free probes, as shown in panel A, was quantified using the AIDA image analyzer software (Raytest, Straubenhardt, Germany). The band intensities of the free probes in the absence of TauR were set as 100%. (C) Unlabeled wild-type *tpa* promoter fragments (P-wt) were used as specific competitor DNA, while mutant *tpa* promoter fragments (P-mut-3/4, carrying the mutations mut-3 and mut-4) (Fig. 4) served as nonspecific competitor DNA. In detail, 0.58 μ M TauR and radioactively labeled wild-type *tpa* promoter fragments were mixed with an 80-, 160-, 320-, 640-, 1,280-, 2,560-, 5,120-, or 10,240-fold excess of unlabeled competitor or noncompetitor DNA. All reactions were performed with 0.6 fmol ³²P-labeled DNA.

tors contain a class I aminotransferase domain binding pyridoxal 5'-phosphate (PLP) (19). Since the putative PLP binding site is highly conserved in TauR (data not shown), we analyzed TauR-mediated gel retardation of the *tpa* promoter in the presence and absence of PLP (Fig. 5A and B). As described above for taurine, PLP did not enhance in vitro binding of TauR to the *tpa* promoter (see "Conclusions" below).

Binding of TauR to the radioactively labeled *tpa* promoter fragment could be reversed by addition of increasing amounts of unlabeled *tpa* promoter DNA, but not by mutant *tpa* promoter DNA (Fig. 5C) (see below), thus demonstrating TauR binding specificity.

TauR binds to direct repeats. The 88-bp BglII-TacI fragment (encompassing the TauR binding site) contains two pairs of almost perfect direct repeats (DR-1a/DR-1b and DR-2a/DR-2b) (Fig. 4). To examine the roles of these sequences in TauR binding, site-directed mutations were created by overlap extension PCR (see Materials and Methods) (Fig. 4). As described above for the wild-type *tpa* promoter, DNA fragments carrying the mutations mut-1 to mut-4 were PCR amplified using the primer pair PJW72-U/PJW73-L (Table 2), radioactively labeled, and incubated with TauR_{Strep}.

The results of DNA mobility shift assays with increasing amounts of TauR_{Strep} are shown in Fig. 6. Binding of TauR to the *tpa* promoter was not influenced by the mutations mut-1 and mut-2 compared to the wild-type promoter. These findings suggest that the direct repeats DR-1a and DR-1b are dispensable for TauR binding. In contrast, binding of TauR was clearly diminished by the mutations mut-3 (in DR-2a) and mut-4 (in DR-2b). Interestingly, the effect of mut-4 was less pronounced than that of mut-3. Since the direct repeats DR-2a and DR-2b differ by 1 base pair (Fig. 4), one may speculate that TauR binds more strongly to DR-2a than to DR-2b. Nonetheless, a combination of both mutations (mut-3/4) completely abolished binding of TauR to the *tpa* promoter, strongly suggesting that both direct repeats, DR-2a and DR-2b, are involved in TauR binding.

Conclusions. *R. capsulatus* TauR was shown to be essential for the activation of *tpa* expression. Since *tauR* is cotranscribed with the *tpa* gene, TauR appears to be a positive autoregulator. Interestingly, in vivo *tpa* expression was clearly enhanced by taurine, but taurine did not influence the binding of TauR to the *tpa* promoter in vitro. An unknown coactivator protein responding to taurine availability and binding to the direct repeats DR-1a/DR-1b immediately upstream of the TauR binding site might be required for responsiveness to taurine.

Alternatively, upon taurine-independent binding of TauR to the *tpa* promoter, TauR might be converted into its transcription-competent form by a reaction between taurine and PLP via the aminotransferase domain of TauR. Like other aminotransferases, TauR might transaminate taurine through conversion of PLP to pyridoxamine 5'-phosphate (PMP). In turn, TauR (carrying PMP) might activate *tpa* transcription. It remains speculative whether conversion of PLP to PMP requires pyruvate or another keto acid as an amino acid acceptor or if amination of PLP in TauR occurs independently of a keto acid. A similar model has been discussed previously for *B. subtilis* GabR, which binds to its target promoter independently of GABA and PLP but requires both GABA and PLP for activation of its target genes (2).

FIG. 6. DNA mobility shift assays with TauR and mutant *tpa* promoter fragments. (A) PCR fragments (269-bp) (marked in Fig. 4) carrying either the wild-type *tpa* promoter or variants mut-1, mut-2, mut-3, mut-4, and mut-3/4 were incubated with increasing amounts of TauR_{Strep} (up to 1.17 μM). (B) The decrease of band intensities for free probes, as shown in panel A, was quantified using the AIDA image analyzer software (Raytest, Straubenhardt, Germany).

Binding of TauR to the *tpa* promoter involves direct repeats spanning the DNA region between -77 and -45 upstream of the transcription start site. Due to the close proximity between the TauR binding site and the putative -35/-10 promoter,

one would expect direct interaction between TauR and RNA polymerase without the necessity of DNA bending.

Like TauR, GabR binds to direct repeats (2). However, the binding sites of the two regulators have different sequences and distances between the repeats. While the centers of the GabR binding sites (ATACCA) are separated by 34 bp (equivalent to three helical turns), the centers of the TauR binding sites (CTGGAC[T/C]TAA) are separated by 23 bp (equivalent to two helical turns). Thus, in both cases, the repeats are located on the same face of the DNA.

Binding of transcriptional regulators to direct repeats is an exception rather than the rule. To our knowledge, GabR from the gram-positive bacterium *B. subtilis* and TauR from the gram-negative bacterium *R. capsulatus* are the only MocR-like regulators that have been experimentally shown to bind to direct repeats. Thus, more work on this widespread but largely uncharacterized family of regulators is needed.

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