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Regulation of testosterone degradation in *Comamonas testosteroni*

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ABSTRACT

Recently, we have identified a gene encoding a LuxR-type factor, TeiR (*Te*stosterone-*i*nducible *R*egulator), which positively regulates steroid degradation in *Comamonas testosteroni*. Herein, we demonstrate that TeiR interacts *in vivo* with steroid catabolic gene promoters. The presence of testosterone induces a significant TeiR protein increase at the early logarithmic phase of growth. Interestingly, it is not until the early stationary phase where the activation of a steroid-inducible gene promoter is observed, indicating that testosterone might not be the true inductor of the steroid degradation pathway. In addition, β-galactosidase expression driven by a testosterone-inducible promoter is prematurely activated in cells cultured in medium supplemented with ethyl acetate extracts obtained from the early stationary phase cell-free supernatants of *C. testosteroni* grown in presence of testosterone. Complementation experiments of *C. testosteroni* wild type performed with *teiR* deletion constructs indicate that extra-copies of deleted-TeiR exert a dominant negative effect on the wild-type TeiR protein. While, when *C. testosteroni teiR* mutants were used to carry out complementation assays only the full length gene can overcome the *teiR* mutant phenotype. Altogether these findings indicate that TeiR regulates steroid catabolic genes interacting with their promoters and suggest that this interaction requires the presence of a testosterone-derived metabolite to induce the system.

1. Introduction

Comamonas testosteroni can grow on a variety of steroid compounds as the sole carbon and energy source [1]. This Gram-negative bacterium can effect the complete oxidative degradation of the steroid skeleton by a series of enzymes, induced by the presence of these compounds in the culture medium [2]. While the genes encoding some of the enzymes catalyzing the oxidoreduction at different positions of the steroid nucleus and the ring opening of the steroid molecule have been identified [3–12], only limited information is available about the mechanisms governing steroid-inducible gene expression. Previously we have identified in *C. testosteroni* ATCC 11996 a gene encoding a LuxR-type factor, named *te*stosterone-*i*nducible *r*egulator (*teiR*), that contains three potentially functional domains including a helix-turn-helix DNA binding domain (aa 327–380), a PAS sensor domain (aa 192–227), and a less conserved N-terminal domain. We demonstrated that *teiR* is required for full expression of *sip48*-β-HSD gene mRNA (encoding a steroid-inducible protein of 48 kDa and 3β-17β-hydroxysteroid dehydrogenase) and also of other steroid degradation genes, including those encoding 3α-hydroxysteroid dehydrogenase (α-HSD), Δ^{5-3}-ketoisomerase, 3-oxo-steroid Δ^1-dehydrogenase, and 3-oxosteroid Δ^4-(5α)-dehydrogenase enzymes [13]. Moreover, we established that *C. testosteroni teiR*-disrupted strains are unable to induce these gene expressions but when *teiR* was provided to the *teiR*-disrupted strain in *trans*, the transcription level of them was restored. Later, a *tesR* gene almost identical to *teiR* was identified in *C. testosteroni* TA441 which regulates three steroid degradation clusters [11]. In addition, two genes coding for negative regulators of α-HSD expression, *repA* and *repB* were identified [14,15]. A recent work determined that TeiR is involved in chemotaxis and mediates steroid sensing and metabolism via its kinase activity [16].

The objectives of the current investigation were to characterize TeiR further determining specific association of TeiR with steroid catabolic gene promoters by DNA–protein cross-linking followed by chromatin immunoprecipitation. In addition, genetic analysis based on *lacZ* transcriptional fusion has been performed to establish the kinetics of steroid gene promoter induction. The findings of the present report demonstrate that TeiR regulates steroid catabolic genes interacting with its promoters and suggest that this interaction requires the presence of a testosterone-derived metabolite to induce the system.

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2. Materials and methods

2.1. Bacterial strains and culture conditions

E. coli was grown at 37 °C in Luria–Bertani (LB) medium [17]. *C. testosteroni* was grown at 30 °C in LB medium or in M9 minimal medium [17] plus sodium acetate (0.2%, w/v), or testosterone (0.28 mg/ml) or both; as indicated in the text. Overnight cultures were diluted 1/100 in fresh medium, incubated 2 h in LB or 12 h in M9, cells were washed, diluted 1/50 in fresh medium and incubated as indicated in each experiment. When needed, antibiotics were added at the following concentrations (in μg/ml): ampicillin, 100; chloramphenicol, 10; gentamycin, 20; kanamycin, 500; spectinomycin, 1300; tetracycline 10.

2.2. Construction of plasmids

The ΔN-terminal and ΔC-terminal TeiR mutants were done by PCR using pGteiR [13] as template and the following primers—ΔNFw: 5′-aaaagcttCGCGCTGACCCTGCATCT-3′; ΔNRv: 5′-aaaagcttTGCTGAGCCAGCTTCACGGGT-3′; ΔCFw: 5′-ggaagcttgctagcATGTGCCCATATTTCGACAC-3′; and ΔCRv: 5′-aagaattcGACCTGACGATCCTGGTTC-3′ to produce the 665 and 588 bp fragments, respectively, with HindIII and EcoRI flanking sites (underlined letters). These PCR fragments were cut with HindIII or HindIII and EcoRI and cloned into the corresponding restriction sites of pBBR1MCS2 [18] generating the deleted N-terminal and C-terminal TeiR constructs, pBΔNTeiR (202–391 residues) and pBΔCTeiR (1–190 residues). The recombinant plasmids were transferred into *C. testosteroni* UT2.5 and *C. testosteroni* UT2.5 *teiR*-mutant strain [13] as described by Pruneda-Paz et al. [12]. DNA sequencing was performed on double-stranded templates derived from pBΔNTeiR and pBΔCTeiR, using an automated DNA sequencer (Macrogen Inc., Seoul, Korea).

2.3. β-Galactosidase assays

The standard procedures described by Miller [19] were used to quantify β-galactosidase activity. The values given throughout this paper represent the average of three independent experiments, each of which was conducted in duplicate samples.

2.4. Conditioned media assays

An initial inoculum of *C. testosteroni* grown in LB plus testosterone for 18 h at 30 °C was centrifuged (12,000 × *g*, 4 min, 25 °C) and filtered (0.45 μm pore size filter). Media was extracted three times with equal volumes of ethyl acetate. The organic phase was evaporated under nitrogen stream and resuspended in methanol. *C. testosteroni* UT2.5 containing the *sip48*-β*hsd::lacZ* fusion was grown in LB plus an aliquot of this methanol extract dissolved in LB (conditioned medium) or testosterone until the indicated OD_{600}, then aliquots of these cultures were assayed for β-galactosidase activity. This strain was previously constructed by chromosomal insertion of a *lacZ* transcriptional fusion containing a steroid-inducible gene promoter (*sip48*-β*hsd::lacZ*) [12].

2.5. TeiR purification and generation of anti-TeiR polyclonal antibody

The *teiR* gene from *C. testosteroni* was previously reported to encode a 391 amino acid polypeptide [13]. The *teiR* coding sequence was placed downstream of the T7 promoter and Shine-Dalgarno region of the plasmid pRSET-C (Invitrogen). *E. coli* BL21(DE3) carrying the resulting plasmid pRSET-*teiR* was grown in LB, and *teiR* expression was induced by isopropyl 1-thio-β-D-galactopyranoside (1 mM). TeiR was purified from the clarified cell extracts by passage through a nickel column as described by the manufacturer (Novagen). SDS-PAGE analysis indicated that a single polypeptide species (>95% purity) was obtained.

For the production of polyclonal anti-TeiR, purified His6-TeiR (100 μg/animal) was emulsified in Freund's complete adjuvant. Two different New Zealand White rabbits were injected intradermally with the antigen preparation. At 2-week intervals, the rabbits received booster doses of the antigen in Freund's incomplete adjuvant. Seven days after the final injection, blood was collected and serum was separated by centrifugation at 1000 × *g* for 20 min at 4 °C. The antibodies were immunopurified from rabbit serum as described [20].

2.6. Western blot analysis

Cultures of *C. testosteroni* wild type and mutant bacteria were grown in LB medium in the presence or absence of testosterone. Cells were harvested by centrifugation and resuspended in SDS-PAGE sample buffer. Proteins were loaded onto a 10% SDS-PAGE gel and electrotransferred to nitrocellulose (Amersham Bioscience UK Limited). The membrane was blocked in PBS containing 0.2% Tween 20 and 5% nonfat dry milk, washed and incubated with the primary anti-TeiR antibody (1:5000) for 1 h at room temperature with shaking. After washing, the blots were incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody (Amersham Bioscience UK Limited) at room temperature for 1 h. Protein–antibody complexes were visualized by an enhanced chemiluminescence detection system (SuperSignal West Pico; Pierce).

2.7. Chromatin immunoprecipitation assays (ChIP)

In all experiments, *in vivo* cross-linking of nucleoproteins was initiated by the addition of formaldehyde (final concentration of 1%) to bacterial cultures. After 30 min, cross-linking was quenched by the addition of glycine (final concentration of 0.5 M). Cells were then harvested from 10 ml of culture by centrifugation, washed twice with PBS, resuspended in 1 ml of lysis buffer (10 mM Tris [pH 8.0], 0.1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride [PMSF], lysozyme 500 mg/ml) and incubated at 37 °C for 15 min. After that, 1% SDS was added and incubated for 5 min. Following lysis, 2.7 ml of immunoprecipitation buffer (50 mM Tris–HCl [pH 7.0], 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1 mM PMSF) were added. DNA was sheared by sonication using a High Intensity Ultrasonic Sonicator (Cole Parmer), 50 W model equipped with a 2 mm tip. The sonication conditions were optimized, three to four sets of 10 s pulses and set to 30% of maximum power, to an average size of 500–1000 bp. Cell debris was removed by centrifugation and the supernatant was retained for use as the input sample in ChIP experiments.

An 800 μl aliquot of the input sample was used for each immunoprecipitation experiment. The sample was incubated with anti-β *E. coli* RNA polymerase antibody (kindly gift from Dr. Ding Jin, National Cancer Institute, Frederick, MD, USA); TeiR rabbit polyclonal antibody; or unrelated antibody for 3 h at room temperature on a rotating wheel. Protein A Sepharose was used to collect the immune complexes. The immunoprecipitates were washed three times with immunoprecipitation buffer, once with immunoprecipitation buffer plus 500 mM NaCl and three times with Tris–EDTA buffer (10 mM Tris–HCl [pH 8.0], 0.1 mM EDTA). Immunoprecipitated samples were uncross-linked by incubation 2 h at 42 °C and 6 h at 65 °C in 0.5 ml elution buffer containing 0.1 M $NaHCO_3$, 1% SDS and NaCl (final concentration 200 mM). Prior to analysis, DNA was

purified from the immunoprecipitates using a PCR purification kit (QIAGEN) and resuspended in 200 μl of water. All ChIP assays were repeated at least twice, and results were found to be reproducible within an error margin of 20%.

Following purification, PCR was used to analyze immunoprecipitated DNA; 2.5 μl DNA samples were used in a 25 μl reaction mix containing a 1 μM of each oligonucleotide primer (*sip48-βhsd* promoter-F 5′ CATTAcccgggCCAGCCCCAGGGGATGAA 3′, and *sip48-βhsd* promoter-R 5′ CGGACggatccGCCTAGTCTCCTTGGATGCA 3′; and 16S rDNA-F 5′ GCCTACGGGAGGCAGCAG 3′ and 16S rDNA-R 5′ ATTACGGCGGCTGCTGG 3′). DNA amplification was catalyzed by Taq DNA polymerase (Invitrogen), and the PCR was allowed to proceed for 25–28 cycles before 5 μl of the reaction was analyzed by electrophoresis on a 7.5% (w/v) polyacrylamide gel.

2.8. Localization of TeiR by immunofluorescence

An overnight culture of *C. testosteroni* wild type or transformed with pBTeiR wt, pBΔCTeiR or pBΔNTeiR plasmids was grown until the exponential phase of growth. A 1 ml sample was taken from this culture and fixed in cold methanol during 10 min. The cells were permeabilized for 7 min with 0.01% Triton X-100 in PBS (PBST). The cells were then rinsed with PBS, blocked with 2.5% normal goat serum in PBST and with 0.2% fish skin gelatin in PBST, and then incubated at room temperature with the primary antibody rabbit purified anti-TeiR (1:5000). Cells were washed with PBST and incubated 1 h with, either Alexa Fluor 488-conjugated donkey anti-rabbit IgG (Molecular Probes, Inc., Eugene, OR). Cells were washed with PBST and chromosomal DNA was counterstained with 4′,6-diamidino-2-phenylindole (DAPI) dye. Slides were mounted in PBS 90% glycerol containing 5 ng/ml p-phenylene-diamine (Sigma–Aldrich). Observations were made with an inverted microscope NIKON Eclipse TE 2000U (Nikon Corporation, Japan).

3. Results

3.1. TeiR interacts in vivo with the steroid degradation gene promoters

In order to examine if TeiR regulates expression of steroid catabolic degradation genes interacting directly with their promoters *in vivo*, we carried out ChIP experiments using bacteria growing either with or without testosterone. Initially we analyzed if TeiR interacts with *sip48-βhsd* promoter using anti-TeiR polyclonal and anti-β *E. coli* RNA polymerase antibodies to immunoprecipitate DNA fragments attached to TeiR and RNA-pol, respectively. Fig. 1 shows PCR analysis by using primers that are specific to *sip48-βhsd* promoter and, as a control, primers for the 16S rDNA fragments. The results clearly show that *sip48-βhsd* promoter is enriched in both the anti-TeiR and anti-β RNA pol immunoprecipitates when the cells were grown in presence of testosterone. To check that these immunoprecipitates were specifically enriched in *sip48-βhsd* promoter DNA (401 bp), the 16S rDNA primers were used to amplify a control region of the chromosome (200 bp). With these primers, the same signal is seen with the anti-TeiR, anti-β RNA pol and control immunoprecipitates. Similar data were observed for *tesB* promoter and for the promoter located between the divergently transcribed *tesA1* and *tesA2* described in *C. testosteroni* TA441 (data not shown) [11]. These results demonstrate that TeiR interacts *in vivo* with steroid degradation gene promoters when testosterone was added to the cultures presumably due to an increased amount of TeiR as well as to a better association of the RNA polymerase β subunit with the promoters resulting in a transcriptional activation.

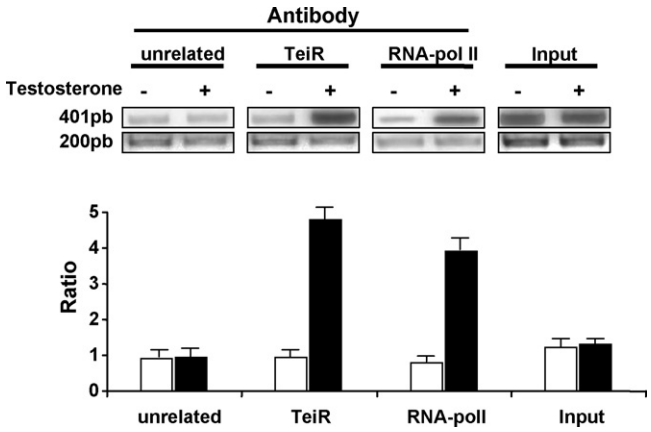

Fig. 1. ChIP analysis of TeiR and RNA polymerase binding to the *sip48* promoter. The figure illustrates the results of a ChIP experiment designed to monitor the binding of TeiR and RNA polymerase to the *sip48-βhsd* promoter in the presence and absence of testosterone. The top panel shows a gel on which PCR products, generated with primers designed to detect either *sip48-βhsd* promoter DNA (401 bp) or control 16S rDNA (200 bp) in each immunoprecipitate (Ab-TeiR, Ab-β RNA polymerase of *E. coli* and an unrelated antibody) were analyzed. The bottom panel is a quantitative representation of the data from three independent experiments. It shows the ratio (with testosterone/without testosterone) of the PCR signals (*sip48-βhsd* promoter DNA: (■) or control 16S rDNA: (□)) in each immunoprecipitate obtained with the different antibodies in both sets of growth conditions. Input corresponds to total DNA before precipitation.

3.2. Monitoring TeiR protein expression during C. testosteroni growth

In order to measure the changes in TeiR levels during *C. testosteroni* growth as well as the *sip48-βhsd* promoter activity, cultures of *C. testosteroni* UT2.5 were grown in LB plus testosterone and, at intervals; aliquots of cultures were removed to measure bacterial growth, TeiR and β-galactosidase levels. The results clearly demonstrate that the expression of the reporter gene controlled by the steroid-inducible promoter occurs in the transition of the logarithmic-stationary phase (Fig. 2A). Western blot assays revealed an increase in TeiR expression, before to the induction of β-galactosidase activity, reaching peak levels at the early logarithmic phase (Fig. 2B). These findings indicate that an early increase in the TeiR level is necessary to induce the steroid assimilation gene promoters.

3.3. Response of steroid-inducible promoter to conditioned medium

In to order to establish if testosterone itself or some testosterone intermediate is the inducer of the *sip48-βhsd* gene we compared the activity of the *lacZ* transcriptional fusion of *C. testosteroni* UT2.5 grown in LB supplemented with testosterone respect to the cells growing in LB supplemented with conditioned medium obtained from the transition logarithmic-stationary phase cell-free supernatants of *C. testosteroni* cultures grown in presence of testosterone. As it is shown in Fig. 3, the addition of the conditioned medium resulted in a premature activation of *sip48-βhsd::lacZ* fusion relative to cells grown in LB plus testosterone, with the most pronounced activation at earlier stages of growth. Interestingly, the initial level of β-galactosidase activity of *C. testosteroni* UT2.5 cells grown in LB supplemented with the conditioned medium was higher than the corresponding cells grown in LB supplemented with testosterone. Altogether these data indicate that the transcription of testosterone-inducible genes is regulated by a

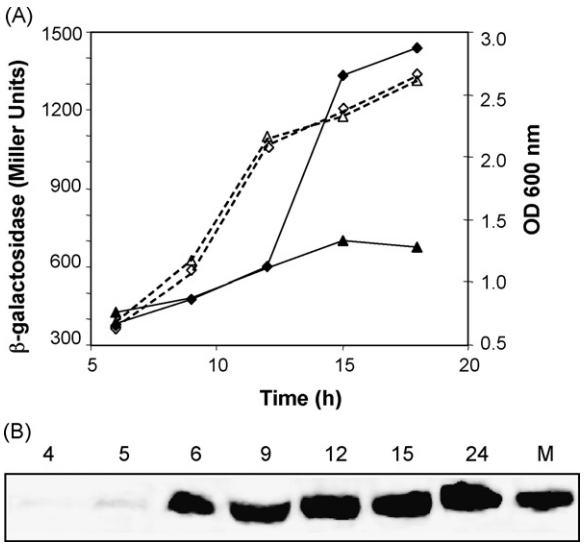

Fig. 2. Monitoring TeiR protein during growth. (A) *Comamonas testosteroni* UT2.5 was grown in LB with (♦) or without (▲) testosterone and at different times aliquots were taken to determine bacterial growth (◊, △) and β-galactosidase levels (♦, ▲). (B) Crude extracts from *C. testosteroni* UT2.5 at the indicated intervals from the exponential phase (4 h) until the stationary phase (24 h) were subjected to SDS-PAGE electrophoresis on 10% gels, transferred to nitrocellulose and probed with polyclonal rabbit anti-TeiR antibody. Detection of bound antibodies was with anti-rabbit HRP-conjugated secondary antibody, followed by the ECL system. The numbers in each lane indicate the time (h) at which the aliquot was taken; in lane M 40 ng of purified TeiR was electrophoresed.

steroid-derived molecule produced when *C. testosteroni* is grown in the presence of testosterone.

3.4. Structure–function analysis of TeiR protein sequence

In order to perform a structure–function analysis of TeiR we examine whether the transformation of *C. testosteroni* UT2.5 with ΔN-TeiR or ΔC-TeiR truncated species alters the *sip48-βhsd*-promoter response to the presence of testosterone. As shown in Fig. 4 the β-galactosidase activity was repressed when this strain received a plasmid encoding either ΔN-TeiR or ΔC-TeiR proteins. On the other hand, the inducible β-galactosidase expression was

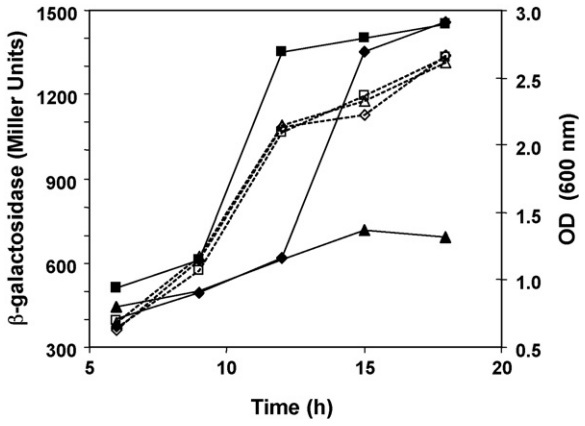

Fig. 3. Effect of conditioned medium on the *sip48-lacZ* transcriptional fusion expression. *C. testosteroni* UT2.5 growing in different experimental conditions was harvested at 6, 9, 12, 15 and 18 h of culture and bacterial growth (□, ◊, △ open symbols) and β-galactosidase activity (■, ♦, ▲) were conducted. The accumulation of β-galactosidase from *sip48::lacZ* fusion in *C. testosteroni* UT2.5 strain growing in LB (▲), LB plus testosterone (♦) or LB supplemented with conditioned medium (■) prepared as described in Section 2 were measured.

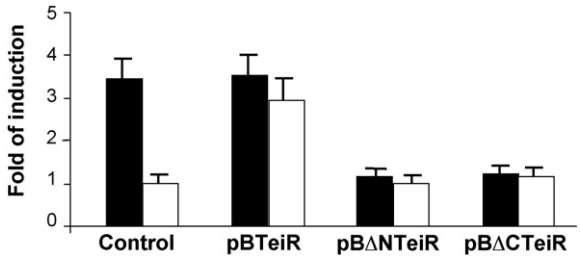

Fig. 4. Structure–function analysis of TeiR sequence. β-Galactosidase activity was measured in *C. testosteroni* UT2.5 (■) and *C. testosteroni* UT2.5 *teiR* mutant (□) transformed with pBTeiR, pBΔNTeiR or pBΔCTeiR. The promoter activity was determined as folds of induction (β-galactosidase activity in the presence of testosterone/β-galactosidase activity in the absence of testosterone) and each value is the average of results from three independent experiments (error bars indicate standard deviations).

not change when *C. testosteroni* UT2.5 was transformed with a plasmid carrying *teiR* wild-type insert. These results suggest that extra-copies of deleted-TeiR exert a dominant negative effect on the wild-type TeiR protein. In addition when *C. testosteroni* UT2.5 TeiR mutant was transformed with the amino- or carboxy-terminal truncated *teiR* versions the inducible β-galactosidase activity was not recovered, indicating that the integrity of TeiR is required for its transcriptional activity (Fig. 4).

Furthermore, the morphological phenotype of *C. testosteroni* UT2.5 transformed with plasmids encoding the wild type and the N- and C-terminal TeiR deletions were analyzed by immunofluorescence assays. The generated antibody was used to examine the TeiR subcellular localization. As shown in Fig. 5A and B a well defined signal was observed in the poles of the cells. When *C. testosteroni* UT2.5 was transformed with a plasmid encoding TeiR wild type or ΔC-TeiR truncated proteins no changes in the morphological aspect and TeiR localization were observed (Fig. 5C and D). In contrast, when *C. testosteroni* UT2.5 was transformed with a plasmid encoding an ΔN-TeiR truncated version an important alteration in the cellular division process was distinguished (Fig. 5E).

4. Discussion

We have previously reported the identification and characterization of TeiR, a LuxR-type transcription factor that is necessary for testosterone degradation in *C. testosteroni* strains [13]. In this work, we have used both genetic and biochemical approaches to study the regulation of the expression of *sip48-βhsd* gene encoding two steroid-inducible proteins. Here, genetic analysis based on *sip48-βhsd::lacZ* transcriptional fusion and ChIP experiments have shown that TeiR interacts on steroid catabolic gene promoters in a growth phase-dependent fashion.

We previously established that *C. testosteroni teiR*-disrupted strains are unable to induce steroid degradation gene expressions but when *teiR* was provided to the *teiR*-disrupted strain in *trans*, the transcription level of them was restored [13]. Here, the complementation experiments of *C. testosteroni* wild type performed with *teiR* deletion constructs indicate that extra-copies of deleted-TeiR exert a dominant negative effect on the wild-type TeiR protein. Meanwhile, when *C. testosteroni teiR* mutant was utilized to carry out complementation assays only the full length gene can overcome the *teiR* mutant phenotype.

It is well known, that bacterial catabolic pathways for aromatic compounds are often arranged as a network of genes or operons coordinately triggered by one or more regulatory proteins, and the effectors of these regulatory proteins are usually either the initial substrates or catabolic intermediates of the pathways. The

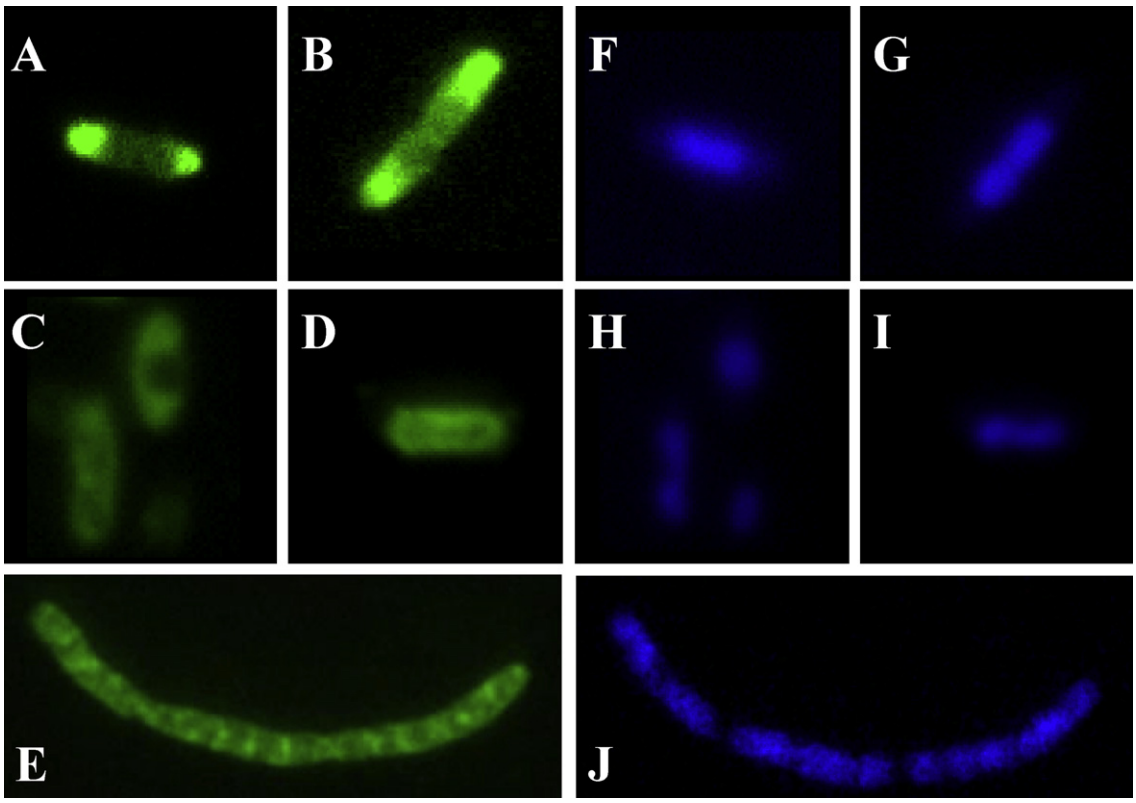

Fig. 5. Subcellular immunolocalization of TeiR. *C. testosteroni* wild type (A and B) and *C. testosteroni* transformed with: pBTeiR wt (C), pBΔCTeiR (D), or pBΔNTeiR (E) were fixed and stained for detection of TeiR with anti-TeiR antibody (green). Panels F–J are corresponding DAPI-stained chromosomal DNA images (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

present results indicate that an early increase in the TeiR level is necessary to induce the steroid assimilation gene promoters. Furthermore, the kinetic analysis of β-galactosidase expression driven by a testosterone-inducible promoter demonstrates that it is prematurely activated in cells cultured in medium supplemented with ethyl acetate extracts obtained from the early stationary phase cell-free supernatants of *C. testosteroni* grown in presence of testosterone. This suggests that a testosterone-derivative metabolite accumulates in batch cultures in the early stationary phase of growth in correlation with TeiR biosynthesis. This result supports the notion that a testosterone degradation intermediate metabolite present in the extracellular compartment can trigger the expression of testosterone-inducible genes. We do not known the molecular nature of the testosterone-derived molecule that accumulates in the extracellular media able to induce steroid catabolic genes although according to the scheme of steroid degradation pathway described by Horinouchi et al. [11], it is possible hypothesize that 9,17-dioxo-1,2,3,4,10,19-hexanorandrostan-5-oic acid or its coenzyme A ester or some other proposed metabolic compound could be the true inductor of the system.

Finally, ChIP experiment results indicate that TeiR interacts *in vivo* with steroid catabolic gene promoters; and testosterone addition to the cultures improves this interaction presumably due to an increased amount of TeiR as well as to a better association of the RNA polymerase β subunit with the *sip48-βhsd* gene promoter resulting in a transcriptional activation. Altogether these findings indicate that TeiR regulates steroid catabolic genes interacting with their promoters and suggest that this interaction requires the presence of a testosterone-derived metabolite to induce the system.

A recent report demonstrated in *C. testosteroni* wild type expressing a TeiR-GFP fusion that TeiR is asymmetrically concentrated at one of the poles of the cell [16]. Moreover, it was reported that TeiR provides swimming and twitching mobility of *C. testosteroni* to the steroid substrate source [16]. Identical TeiR subcellular localization was observed using an anti-TeiR antibody in a *C. testosteroni* UT2.5 or in bacteria transformed with a plasmid encoding a ΔC-TeiR truncated version. In contrast, when *C. testosteroni* UT2.5 was transformed with a plasmid encoding an ΔN-TeiR truncated protein an important alteration in the cellular division process was distinguished. These data suggest that in addition to the regulatory role, TeiR may participate in cellular division events.

The observation that α-HSD gene expression is controlled through a de-repression mechanism in which the binding of two repressor proteins (RepA and RepB) is prevented by testosterone [15] and our previous reported data that indicate the complete lack of α-HSD gene transcription when the *teiR*-disrupted mutant was grown in the presence of testosterone [13] suggest that a complex mechanism controls steroid gene expressions.

Taken together these findings it is possible to hypothetize that the assimilation of steroid compounds in *C. testosteroni* may start by a steroid-dependent gene promoter de-repression allowing the accumulation of a steroid-derived molecule. Finally, TeiR, interacting with these promoter genes and this testosterone-derivative metabolite, triggers the complete induction of steroid catabolic genes.

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