Crystal Structure of the Quorum-sensing Protein TraM and Its Interaction with the Transcriptional Regulator TraR*

Received for publication, February 19, 2004, and in revised form, March 22, 2004
Published, JBC Papers in Press, March 24, 2004, DOI 10.1074/jbc.M401855200

Alessandro Vannini, Cinzia Volpari, and Stefania Di Marco*
From the Department of Biochemistry, Istituto di Ricerche di Biologia Molecolare Pietro Angeletti, 00040 Pomezia, Rome, Italy

Transfer of the tumor-inducing plasmid in Agrobacterium tumefaciens is controlled by a quorum-sensing system whose main components are the transcriptional regulator TraR and its autoinducer. This system allows bacteria to synchronize infection of the host plant when a “quorum” of cells has been reached. TraM is an A. tumefaciens protein involved in the regulation of this system because it binds to TraR and prevents it from binding DNA. As a first step to understanding the molecular basis for the regulation of TraR by TraM, we have determined the crystal structure of TraM at 1.65 Å resolution. This protein is packed as a dimer, with each monomer consisting mainly of two antiparallel α helices. Monomers are tightly associated, with a large hydrophobic area buried upon dimerization. Secondly, we characterized the TraR-TraM complex in vitro. TraM (11.4 kDa, monomer molecular mass) binds tightly TraR (27 kDa, monomer molecular mass) forming a stable oligomeric complex that likely accounts for two TraR and two TraM dimers.

Quorum-sensing is a term that reflects the ability of bacteria to control the expression of specific operons in a cell density-dependent manner and is based on the production, release, and “sensing” of small signal molecules called autoinducers that accumulate in the environment as a function of cell density. Both Gram-negative and Gram-positive bacteria use it to regulate several physiological functions, including bioluminescence, virulence factor expression, antibiotic production, and biofilm development (1).

As the primary cause of the crown gall disease in plants, the Gram-negative bacterium Agrobacterium tumefaciens is responsible for substantial loss of perennial crops worldwide (2). Pathogenesis involves transfer of the tumor-inducing plasmid from the bacterium to the host cell nucleus and the subsequent transcription of opines and phytohormones, which cause large tumors to form on stems (3). The conjugal transfer of the tumor-inducing plasmid is strictly controlled by a LuxR-type quorum-sensing circuit (1). This system is composed of the activator TraR (homologous to LuxR), a cis-acting DNA inverted repeat called tra box, and the autoinducer, N-(3-oxooctanoyl)-l-homoserine lactone. The autoinducer, synthesized by the TraI protein (homologous to LuxI), diffuses freely between the cellular and external environment and, after accumulation, activates the transcriptional regulator TraR. The TraR-autoinducer complex binds to tra boxes, upstream of three different tra operons to stimulate transcription of the conjugal genes in a positive feedback loop (4, 5).

Recently, the crystal structure of TraR bound to its autoinducer and to DNA has been reported (6, 7). TraR binds the DNA as a dimer. Each monomer is composed of an N-terminal domain that binds the ligand in an enclosed cavity and a C-terminal domain that binds DNA via a helix-turn-helix motif. Additional regulatory proteins modulate the activity of LuxR-type proteins, such as the protein TrlR, a defective LuxR homologue that forms inactive heterodimers with TraR (8), and other regulators like QscR and TraM that form inhibitory complexes with LuxR-type proteins (9, 10). Among these, the tumor-inducing plasmid-encoded protein TraM from A. tumefaciens modulates TraR-dependent transcriptional activation by direct protein-protein interaction. TraM acts as an anti-activator of TraR by binding to its C-terminal domain and therefore prevents TraR from binding DNA (9, 11, 12). This inhibition is required for the normal operation of the quorum-sensing pathway; as a consequence, null mutations in TraM result in constitutive conjugal even at low population density (13). TraM acts either to prevent TraR from initiating transcription of the tra regulon under non-inducing condition or to shut it down efficiently when the opine signal is no longer available during infection (14). Thus, TraM plays a key role in determining the threshold level of the bacterial population required for initiating the tumor-inducing plasmid conjugal transfer (9). We have determined the crystal structure of the recombinant TraM protein. The structure reveals two molecules per asymmetric unit, arranged as a dimer. To understand the molecular basis of TraR-TraM interaction, we have reconstituted and characterized the complex in vitro. Dimeric TraM binds tightly to dimeric TraR with an equimolar ratio, forming a stable oligomeric complex of ~150 kDa. A model of the TraR-TraM complex is proposed.

EXPERIMENTAL PROCEDURES

Protein Production, Crystallization, and Data Collection—TraR and TraM were purified from Escherichia coli as described previously (15, 16). TraM crystallization has been described elsewhere (16). Data from TraM crystals were collected at 100 K using synchrotron radiation at the beamline ID29, European Synchrotron Radiation Facility (ESRF), Grenoble. Diffraction data were processed with MOSFLM (17) and scaled with SCALA (Ref. 18, Table I).

Structure Determination and Refinement—Selenium atoms were located using the program SnB (19) as described previously (16). Heavy atom refinement and density modification were carried out at 2.0 Å resolution, using multiwavelength anomalous diffraction data from the peak and the remote (as the reference wavelength), in CNX (Accelrys; PharmaCode Inc.). 60% of the model was built using QUANTA 2000 (Accelrys; PharmaCode Inc.). The model was then fed into Arp/Warp (modality WarpNTrace) (20) to combine model building with iterative
Crystal Structure of TraM and Its Interaction with TraR

| Table I | Summary of TraM x-ray diffraction data and refinement statistics |
|----------------|------------------------------------------------------------|
| **X-ray diffraction data** | Native | Se MAD | Peak |
| Space group | P21212 | P21212 |   |
| Cell constants (Å) |   |   |   |
| a | 77.809 | 76.436 |   |
| b | 47.432 | 47.097 |   |
| c | 47.621 | 47.469 |   |
| Wavelength | 0.9611 | 0.9611 | 0.9791 |
| Resolution (Å)* | 50–1.65 (1.67–1.65) | 50–1.8 (1.91–1.80) | 50–2.0 (2.12–2.00) |
| R cryst | 5.2 (33.3) | 3.6 (22.4) | 4.6 (18.5) |
| (Rfree)** | 6.9 (2.2) | 13.9 (5.3) | 9.8 (3.4) |
| No. observations | 247,930 | 161,543 | 130,145 |
| No. unique observations | 201,909 | 15,965 | 11,970 |
| Completeness (%) | 98.6 (92.3) | 96.9 (83.5) | 99.3 (99.6) |

| Refinement statistics |   |   |
| Resolution (Å) | 50–1.65 | 50–1.80 |
| R-factor ¹ | 0.21 | 0.18 |
| R-free ² | 0.26 | 0.21 |
| R.m.s.d. bond lengths (Å) | 0.026 | 0.034 |
| R.m.s.d. bond angles (Å) | 2.004 | 2.197 |
| Overall B-factor (Å²) | 19.8 | 20.5 |
| ϕ, ψ angle distribution ³ |   |   |
| In core region | 153 (95.1) | 156 (96.9) |
| In allowed region | 8 (4.9) | 5 (3.1) |
| In disallowed region | 0 (0) | 0 (0) |
| Number of atoms | 1503 | 1490 |
| Protein | 219 | 129 |
| Solvent |   |   |

¹ Highest resolution of data set with highest resolution bin in parentheses.
² Defined as mean [I] / S.D.
³ R.m.s.d. is the root mean square deviation from ideal geometry.
⁴ As defined by PROCHECK (30), the percentage distribution is given in parentheses.

Structure refinement, using REFMAC (21) between 50 and 1.8 Å resolution. This results in an improvement of the electron density maps that allowed us to build over 90% of the model after two runs (200 cycles each) of ARP/WARP, each followed by manual rebuilding. The final model was then refined against the native dataset between 50 and 1.65 Å, using REFMAC (21). Refinement statistics are listed in Table I. Figures were generated with PyMOL (DeLano Scientific). Electrostatic calculations and associated figures were performed with GRASP (22).

Size-exclusion Chromatography—To form the TraR-TraM complex, variable amounts of purified TraR (0.5 mM) and TraM (1.6 mM) were mixed and incubated for 12 h at 4 °C in buffer A, containing 20 mM Tris-HCl, pH 8.0, 200 mM NaCl, 10% glycerol, 1 mM dithiothreitol. Samples were loaded on a Superdex 200 10/30 column (Amersham Biosciences) equilibrated with buffer A. Elution was performed at 4 °C, and the flow-rate was held at 0.20 ml/min. The column was calibrated using markers of known molecular weights (catalogue number 1511901; Bio-Rad).

Dynamic Light Scattering (DLS)¹ and Isothermal Titration Microcalorimetry (ITC) — DLS measurements were performed using a DynaPro-801 instrument with temperature control (Protein Solutions). Protein samples were in buffer 50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 10% glycerol, 1 mM dithiothreitol. For DLS measurements, the concentrations of isolated TraR and TraM were 320 nM and 1.1 mM, respectively. The concentration of the TraR-TraM complex used for DLS measurements was 150 μM. Data analyses were performed on DLS analysis software (Protein Solutions). Measurements were done at 20 °C.

An Omega titration calorimeter (MicroCal) was used for calorimeter measurements. Titrations were performed at 25 °C and consisted of 17 injections of 15 μl each made every 240 s into a reaction cell volume of 1.296 ml. All proteins were in dialysis equilibrium with buffer 50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 10% glycerol, 1 mM dithiothreitol. TraR, 170 μM, was titrated into 15 μM TraM. Thermodynamics parameters for the binding reaction were derived using ORIGIN ITC software (MicroCal) by fitting the corrected binding isotherm to a single-site binding model, with stoichiometry (N), enthalpy (ΔH°), and equilibrium dissociation constant (Kd) allowed to float during non-linear least-squares fit of the data.

RESULTS AND DISCUSSION

Three-dimensional Structure of TraM—Both refined x-ray structures of native TraM at 1.65 Å (Protein Data Bank number 1US6) and the seleno-methionine-labeled TraM at 1.8 Å resolution (Protein Data Bank number 1UPG) contain two TraM molecules, A and B, per asymmetric unit, packed as a dimer (Table I). The two structures are identical and superimpose with a root mean square deviation of 0.19 Å for Ca atoms. The final model includes 189 of 204 residues for the dimer, and the structure description will focus on the higher resolution structure. In molecule A, electron density is missing for the first 11 residues and the last one, whereas in molecule B only the last three residues are disordered. A portion of the electron density is shown in Fig. 1A.

Each monomer presents a T-shaped structure of dimensions ~45 Å × 27 Å × 12 Å and can be divided into two parts, a “globular head” and an “elongated tail” (Fig. 1, B and D). The two monomers are identical and superimpose with a root mean square deviation of 0.4 Å for Ca atoms. The core of the molecule is composed of two antiparallel α-helices, α2 (residues 26–53) and α4 (residues 66–94) that pack to each other with a 20° angle, consistent with a “ridge in groove” model for helical packing (23) where the angle between axes of adjacent helices can be either 20° or 50°. Helices α2 and α4 and the short helix α3 (residues 55–60) form the elongated tail. The globular head is formed by the juxtaposition of the helix α1 (residues 15–22) and the N and C termini over the end portion of the long helices α2 and α4. The N and C termini interact with each other via two short antiparallel β-strands, β1 (residues 3–6) and β2 (residues 96–98), to form a small β-sheet. In monomer A, only the strand β2 is visible and stabilized by interactions with a symmetry-related molecule.

¹ The abbreviations used are: DLS, dynamic light scattering; ITC, isothermal titration microcalorimetry; Vr, retention volume.
Each monomer intertwines with the other in a "head-to-tail" fashion, forming a very compact dimer of dimensions $47 \times 35 \times 20$ Å (Fig. 1B). Contacts between monomers involve a large part of each chain. The long helices $\alpha 4$ from each monomer are antiparallel and touch each other to form a $50^\circ$ angle, once again consistent with a ridge in the groove model of helix packing (23). Helix $\alpha 1$ and the N-terminal portion of helix $\alpha 2$ contact the C-terminal half of helix $\alpha 4$ on the other monomer. As a result, two distinct surfaces exist in the dimer; the front one is irregular, being formed by two adjacent globular heads flanked by two narrow elongated tails, whereas the other on the back side of the molecule is relatively flat (Fig. 1D).

TraM can be classified as an $\alpha$-domain structure (Fig. 1C) (24, 25). Searching with MATRAS (26), a program for protein structure comparison, in the non-redundant protein structure database did not highlight any significant similarity using either the monomer or the entire dimer as a search model. Only local structural similarity was found for the antiparallel helices $\alpha 2$ and $\alpha 4$. The highest structural similarity ($Z$ score = 10, root mean square deviation of 1.1 Å for 57 aligned Ca atoms) is with the small repressor Rop from E. coli (Protein Data Bank number 1NKD; Ref. 27), which forms a typical four-helix bundle upon dimerization but with a helix packing architecture different from TraM.

An extensive contact area is present between monomers, burying 1100 Å² of surface area, representing 20% of total surface area of the monomer. The TraM interface consists of 25% polar and 75% non-polar residues (Fig. 1D). Although surface interactions between monomers are predominantly hydrophobic, polar interactions include three direct and nine water-mediated hydrogen bonds. It has been observed that obligate homodimers contain relatively few intermolecular hydrogen bonds (28). The association in the TraM homodimer displays characteristics of other non-transient complexes (28), and the two monomers twist together across the interface with a high complementarity. Taken together, these findings suggest that in TraM the monomer-to-monomer association is tight and specific, giving rise to an obligatory dimer.

Extensive mutagenesis analyses have been carried out on TraM (9, 11). To understand the molecular basis of the interactions of TraR with TraM, we have mapped on the TraM surface only those residues whose alanine mutants have demonstrated a reduction in TraR binding efficiency as well as a loss of activity in vivo: Leu-29, His-40, Arg-41, Leu-54, Tyr-72, Val-86, Gly-94, and Pro-97 (9, 11) (Fig. 2, A and B).

Remarkably, all of these residues face exclusively the front side of the dimer on a discrete grooved surface formed by the elongated tail and an adjacent small region of the globular head in each monomer (Fig. 2, A and B). None of these residues is involved in the dimeric interface, suggesting that TraM could interact with TraR in the dimeric form seen in the crystal structure.

---

**Fig. 1.** A, 2 $F_o - F_c$ electron density map of the refined TraM structure (1.65 Å, 1.2σ) around residues 72–96. Oxygen, carbon, sulfur, and nitrogen are colored red, yellow, green, and blue, respectively. Note Met-79 and Met-83, both refined as double conformers. B, ribbon stereo diagram of the TraM dimer. The two subunits, referred to as molecules A and B in the text forming the homodimer are shown in magenta and yellow, respectively. C, topology diagram of the TraM dimer. Color coding as in panel B. D, electrostatic surface of TraM monomer B, with blue and red indicating basic and acidic regions, respectively. The monomer A is represented as a magenta ribbon. Two orthogonal views are shown.
Characterization of TraR-TraM Complex—A unique symmetrical peak eluted at a retention volume ($V_e$) of 12.9 ml when equimolar concentrations of TraR and TraM were applied onto the size-exclusion chromatography column (Fig. 3A, peak 1). This peak was resolved into two bands on SDS-PAGE, migrating at the position expected for each protein with no evidence of proteolytic degradation (Fig. 3A, inset). The elution profile of the complex was compared with those of the isolated proteins (Fig. 3A, peaks 2 and 3). TraR eluted as a single symmetrical peak at 15.3 ml corresponding to 55 kDa, which represents the dimeric form of TraR (7, 15, 29) in agreement with DLS data (Table II). Isolated TraM, 11.4 kDa molecular mass for the monomer, eluted as a single symmetrical peak at 16.0 ml corresponding to 30 kDa, in agreement with previous biochemical studies (9) and in a concentration-independent manner in the range 0.05–2.5 mM. The same estimated value was obtained by DLS experiment (Table II). This value is slightly higher than the theoretical value for dimeric TraM but could be well explained taking into account the elongated dimeric structure of TraM. If the shape of the protein (or oligomer) is nonspherical, its molecular mass from molecular sizing (Fig. 3A, peak 3) and DLS (Table II) can be overestimated due to a higher translation frictional coefficient and hence a lower translational diffusion coefficient than for a spherical particle of the same molecular mass. The retention volume for the peak of the complex corresponds to a 155-kDa protein (Fig. 3A, peak 1), which matches an oligomer formed by two TraR dimers and two TraM dimers. In our hands, the size-exclusion chromatography behavior of the individual purified proteins and the complex was unaffected by lowering or increasing the protein concentrations (0.05–0.5 mM). The complex always resulted in an oligomer of 155 kDa, whereas TraR and TraM always elute as dimers (data not shown). These data were confirmed by DLS (Table II) and disagree with previously published data in which the molecular mass of the complex, formed by co-incubation of E. coli extracts containing TraR and His$_6$TraM, was estimated as 60 kDa by size-exclusion chromatography (9). In the same study, E. coli extracts containing radiolabeled TraM had always a smaller degradation product. The authors also stated that isolated proteins eluted as higher molecular mass complexes when higher protein concentrations were used and that co-expression of TraR and His$_6$TraM in E. coli from a single plasmid revealed a protein peak that eluted at high molecular mass (9).

The binding affinity and stoichiometry of TraR-TraM interaction were determined using ITC (Fig. 3C). The fit to the integrated binding isotherm indicates a dissociation affinity constant, $K_d = 49$ nM and essentially a 1:1 stoichiometry (calculated value for TraR-TraM of $n = 0.851$). The positive value of enthalpy ($+6.174$ Kcal/mol) indicates an endothermic reaction, where hydrophobic interactions drive the complex formation. The difference between the affinity constant calculated...
from the rate constants obtained by surface plasmon resonance (1–4/\text{H}11003/1000/2/9\text{M}) (9) and that determined by ITC (49/\text{H}11003/1000/2/9\text{M}) could largely be because of differences in the type of assay.

To assess the ability of TraM to abolish the DNA binding activity of TraR upon complex formation, two excesses of \textit{tra}\textit{box}, a specific DNA 18-base pairs operator that binds TraR (29), were incubated with the TraR-TraM complex and then loaded onto a size-exclusion chromatography column. The elution profile of the sample was compared with that of TraR, \textit{tra}\textit{box}, and the TraR-\textit{tra}\textit{box} complex (Fig. 3B). Although isolated TraR is able to bind \textit{tra}\textit{box}, producing a shift of the protein peak from \(V_e = 15.3\) ml to 14.7 ml, the TraR-TraM complex is not able to bind \textit{tra}\textit{box}, resulting in two separate peaks, one corresponding to the complex (\(V_e = 12.9\) ml) and the other to the isolated \textit{tra}\textit{box} (\(V_e = 16.6\) ml) (Fig. 3B).

Model of TraR-TraM Complex—The crystal structure of TraM shows that all TraM mutants with an impaired ability in binding TraR (9, 11) are exposed and distributed only along the front grooved surface of the dimer (Fig. 2, A and B). Mutagenesis analysis of TraR has shown that residues crucial for TraM binding are all located in the DNA binding domain (6, 7, 12). Thus, for clarity, we have considered just the DNA binding domain of TraR in the description of a possible mode of interaction.

A dimer-to-dimer interaction between TraR and TraM does not seem plausible. Based on crystal structures of TraR (6, 7) and TraM (this study), not all TraM residues crucial for TraR binding would be in contact simultaneously with one dimeric DNA binding domain of TraR. Furthermore, the presence of two globular heads in the TraM dimer (Fig. 2B) provides a steric hindrance that would not allow TraR to bind into the two grooves where most of TraM mutants are located. Lastly, this hypothesis does not agree with size-exclusion chromatography and DLS results (Fig. 3A and Table II). Instead, a complex formed by two TraR dimers and two TraM dimers would account for our biochemical results and agree with previous studies that suggest that the association of TraM and TraR is more complex than a simple one-to-one binding (9). Assuming no relevant conformational changes upon complex formation, a

---

**TABLE II**

| TraR, TraM, and TraR-TraM complex: estimation of molecular mass |
| --- |
| \(R_H\)a | Molecular massb |
| nm | kDa |
| TraR | 3.46 | 59 |
| TraM | 2.61 | 30 |
| TraR-TraM complex | 5.2 | 157 |

a Mean hydrodynamic radius derived from the measured translational diffusion coefficient using the Stokes-Einstein equation.
b Molecular mass estimated from \(R_H\) assuming that the particles are spherical and of standard density.

---

from the rate constants obtained by surface plasmon resonance (1–4/\text{H}11003/1000/2/9\text{M}) (9) and that determined by ITC (49/\text{H}11003/1000/2/9\text{M}) could largely be because of differences in the type of assay.

To assess the ability of TraM to abolish the DNA binding activity of TraR upon complex formation, two excesses of \textit{tra}\textit{box}, a specific DNA 18-base pairs operator that binds TraR (29), were incubated with the TraR-TraM complex and then loaded onto a size-exclusion chromatography column. The elution profile of the sample was compared with that of TraR, \textit{tra}\textit{box}, and the TraR-\textit{tra}\textit{box} complex (Fig. 3B). Although isolated TraR is able to bind \textit{tra}\textit{box}, producing a shift of the protein peak from \(V_e = 15.3\) ml to 14.7 ml, the TraR-TraM complex is not able to bind \textit{tra}\textit{box}, resulting in two separate peaks, one corresponding to the complex (\(V_e = 12.9\) ml) and the other to the isolated \textit{tra}\textit{box} (\(V_e = 16.6\) ml) (Fig. 3B).

Model of TraR-TraM Complex—The crystal structure of TraM shows that all TraM mutants with an impaired ability in binding TraR (9, 11) are exposed and distributed only along the front grooved surface of the dimer (Fig. 2, A and B). Mutagenesis analysis of TraR has shown that residues crucial for TraM binding are all located in the DNA binding domain (6, 7, 12). Thus, for clarity, we have considered just the DNA binding domain of TraR in the description of a possible mode of interaction.

A dimer-to-dimer interaction between TraR and TraM does not seem plausible. Based on crystal structures of TraR (6, 7) and TraM (this study), not all TraM residues crucial for TraR binding would be in contact simultaneously with one dimeric DNA binding domain of TraR. Furthermore, the presence of two globular heads in the TraM dimer (Fig. 2B) provides a steric hindrance that would not allow TraR to bind into the two grooves where most of TraM mutants are located. Lastly, this hypothesis does not agree with size-exclusion chromatography and DLS results (Fig. 3A and Table II). Instead, a complex formed by two TraR dimers and two TraM dimers would account for our biochemical results and agree with previous studies that suggest that the association of TraM and TraR is more complex than a simple one-to-one binding (9). Assuming no relevant conformational changes upon complex formation, a
feasible architecture would be that shown in Fig. 4, A and B, in which each TraR dimer located externally contacts two TraM dimers simultaneously, acting as a clamp. A model in which each dimer of one protein simultaneously contacts two dimers of the other protein is the only one possible for a stable two-to-two dimer complex. This model implies that the 2-fold axis of each TraR dimer is orthogonal to the 2-fold axis of each TraM dimer (Fig. 4A). Using the crystal structures of the isolated proteins, we constructed manually, imposing geometrical constraints shown in Fig. 4A, a three-dimensional arrangement of a putative complex that displays a good shape complementary (Fig. 4B). Here, each narrow groove of one TraM dimer seems to accommodate well the recognition helix of one TraR DNA binding domain, mimicking a half-site of the DNA operator. In addition, the surface charge distribution does not reveal any relevant repulsion between single components (data not shown). The N terminus of TraM lies very close to the TraR binding region, and our model suggests that an extended N-terminal TraM variant, like a His-tagged protein (9), might interfere with the formation of a stable oligomeric complex with TraR (Fig. 4B).

The present work clarifies some issues raised from previous biochemical studies of the TraR-TraM complex and suggests, based on the three-dimensional structure of the isolated proteins, a possible mode of interaction that leads to the complete inactivation of the key quorum-sensing regulator TraR in the pathogenic bacterium _A. tumefaciens_.

Acknowledgments—We thank Bill Shepard for assistance in the multiwavelength anomalous diffraction (MAD) data collection at beamline ID29, European Synchrotron Radiation Facility, Grenoble, Andrea Carfi and Raffaele De Francesco for discussions, and Janet Clench for revision of the manuscript.

REFERENCES

1. Miller, M. B., and Bassler, B. L. (2001) _Annu. Rev. Microbiol._ **55**, 165–199
2. Zhu, J., Oger, P. M., Schrammeier, B., Hooyskaas, P. J., Farrand, S. K., and Winans, S. C. (2000) _J. Bacteriol._ **182**, 3885–3895
3. Farrand, S. K. (1998) in _The Rhizobiales, Molecular Biology of Model Plant-associated Bacteria_ (Spanik, H. P., Kondorosi, A., and Hooykaas, P. J. J., eds) pp. 199–233, Kluwer Academic, Dordrecht, The Netherlands
4. Fuqua, C., and Winans, S. C. (1996) _Mol. Microbiol._ **20**, 1199–1210
5. Fuqua, C., Beck von Bodman, S., Hawes, J., and Farrand, S. K. (1999) _Mol. Microbiol._ **32**, 1077–1089
6. Vannini, A., Volpari, C., Gargioli, C., Muraglia, E., Courtese, R., De Francesco, R., Neidemann, P., and Di Marco, S. (2002) _EMBO J._ **21**, 4395–4401
7. Zhang, R. G., Pappas, T., Brace, J. L., Miller, P. C., Oulmassov, T., Molyneaux, J. M., Anderson, J. C., Bashkin, J. K., Winans, S. C., and Joachimiak, A. (2002) _Nature_ **417**, 971–974
8. Chai, Y., Zhu, J., and Winans, S. C. (2001) _Mol. Microbiol._ **40**, 414–421
9. Swiderska, A., Berndtson, A. K., Cha, M. R., Li, L., Beaudouin, G. M., Zhu, J., and Fuqua, C. (2001) _J. Biol. Chem._ **276**, 49449–49458
10. Ledsham, F., Ventré, I., Soscia, C., Foglino, M., Sturgis, J. N., and Landzuka, A. (2003) _Mol. Microbiol._ **48**, 199–210
11. Hwang, I., Smyth, A. J., Luo, Z. Q., and Farrand, S. K. (1999) _Mol. Microbiol._ **34**, 282–294
12. Luo, Z. Q., Qin, Y., and Farrand, S. K. (2000) _J. Biol. Chem._ **275**, 7713–7722
13. Piper, K. R., and Farrand, S. K. (2000) _J. Bacteriol._ **182**, 1080–1088
14. Luo, Z. Q., Su, S., and Farrand, S. K. (2003) _J. Bacteriol._ **185**, 5665–5672
15. Vannini, A., Volpari, C., Gargioli, C., Muraglia, E., De Francesco, R., Neidemann, P., and Di Marco, S. (2002) _Acta Crystallogr. Sect. D Biol. Crystallogr._ **58**, 1362–1364
16. Vannini, A., Volpari, C., and Di Marco, S. (2004) _Acta Crystallogr. Sect. D Biol. Crystallogr._ **60**, 146–148
17. Leslie, A. G. W., Brick, P., and Wonacott, A. T. (1986) _Daresbury Lab. Inf. Quart. Protein Crystallogr._ **18**, 33–39
18. Evans, P. R. (1993) in _Proceedings of CCP4 Study Weekend. Data Collection and Processing_ (Sawyer, L., Isaacs, N., and Bailey, S., eds) pp. 114–122, Warrington, Daresbury Laboratory, Cheshire, UK
19. Weeks, C. M., and Miller, R. (1999) _Acta Crystallogr. Sect. D Biol. Crystallogr._ **55**, 492–500
20. Perrakis, A., Morris, R. J. H., and Lamzin, V. S. (1999) _Nature Struct. Biol._ **6**, 468–463
21. Murshudov, G., Vainog, A. A., and Dodson, E. J. (1997) _Acta Crystallogr. Sect. D Biol. Crystallogr._ **53**, 240–255
22. Nicholls, A., Sharp, K., and Honig, B. (1991) _Proteins_ **11**, 283–296
23. Chotia, C., Levitt, M., and Richardson, D. (1981) _J. Mol. Biol._ **155**, 215–250
24. Conlin, T., Brenner, S. E., Hubbard, T. J. P., Chothia, C., and Murzin, A. (2002) _Nucleic Acids Res._ **30**, 264–267
25. Murzin, A. G., Brenner, S. E., Hubbard, T., and Chothia, C. (1995) _J. Mol. Biol._ **247**, 536–540
26. Kawabata, T., and Nishikawa, K. (2000) _Proteins_ **41**, 108–122
27. Vlassi, M., Dauter, Z., Wilson, K. S., and Kokkinidis, M. (1998) _Acta Crystallogr. Sect. D Biol. Crystallogr._ **54**, 1245–1253
28. Jones, S., and Thornton, J. M. (1995) _Prog. Biophys. Mol. Biol._ **63**, 31–65
29. Zhu, J., and Winans, S. C. (1999) _Proc. Natl. Acad. Sci. U. S. A._ **96**, 4832–4837
30. Laskowsky, R. A., MacArthur, M. W., Moss, D. S., and Thornton, J. M. (1999) _J. Appl. Crystallogr._ **32**, 283–291
Crystal Structure of the Quorum-sensing Protein TraM and Its Interaction with the Transcriptional Regulator TraR
Alessandro Vannini, Cinzia Volpari and Stefania Di Marco

J. Biol. Chem. 2004, 279:24291-24296.
doi: 10.1074/jbc.M401855200 originally published online March 24, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M401855200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 28 references, 7 of which can be accessed free at http://www.jbc.org/content/279/23/24291.full.html#ref-list-1