Crystal Structure and Function of the Zinc Uptake Regulator FurB from *Mycobacterium tuberculosis* 

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Members of the ferric/zinc uptake regulator (Fur/Zur) family are the central metal-dependent regulator proteins in many Gram-negative and -positive bacteria. They are responsible for the control of a wide variety of basic physiological processes and the expression of important virulence factors in human pathogens. Therefore, Fur has gathered significant interest as a potential target for novel antibiotics. Here we report the crystal structure of FurB from *Mycobacterium tuberculosis* at a resolution of 2.7 Å, and we present biochemical and spectroscopic data that allow us to propose the functional role of this protein. Although the overall fold of FurB with an N-terminal DNA binding domain and a C-terminal dimerization domain is conserved among the Zur/Fur family, large differences in the spatial arrangement of the two domains with respect to each other can be observed. The biochemical and spectroscopic analysis presented here reveals that *M. tuberculosis* FurB is Zn(II)-dependent and is likely to control genes involved in the bacterial zinc uptake. The combination of the structural, spectroscopic, and biochemical results enables us to determine the structural basis for functional differences in this important family of bacterial regulators.

Iron, zinc, and other transition metals are essential elements for almost all living organisms as they play important roles in a wide range of cellular processes (1, 2). They act either as structural components or as obligate co-factors in various functions ranging from respiration to DNA replication. A balanced efflux/influx of these metal ions has to be achieved to satisfy the requirements of the cell and to avoid toxicity. Bacteria have therefore developed highly sophisticated systems to reach homeostasis based on storage, export, and uptake of metals (3–6). Furthermore, the regulation of gene expression as a response to changing conditions in the environment is one of the key elements in survival of any bacterial pathogen in the host. Unraveling the molecular basis of regulatory networks is therefore of great importance to understand virulence and pathogenesis (7, 8).

The ferric uptake regulator (Fur) family is one of the crucial components necessary to activate or repress this articulated and complex machinery. Members of this family regulate uptake systems for iron and sometimes also for manganese and zinc (9–12). They act on a transcriptional level by binding the operon regions of Fur-regulated genes. Once the protein is bound to its target DNA, it blocks access of the RNA polymerase, thereby repressing the downstream genes. Their homodimeric nature allows DNA interaction mainly with palindromic or pseudo-palindromic regions, where a helix-turn-helix protein domain is located in the major groove of the oligonucleotide (13). Fur and zinc uptake regulator (Zur) belong to the same family showing overall sequence similarities but evidently dissimilar biological functions (10). Fur has been characterized as a generic iron-responsive regulator. In many bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa*, members of this family are also involved in the control of a variety of metabolisms as follows: iron uptake and storage, defenses against oxidative and acid stress, as well as the regulation of a number of genes responsible for virulence, including hemolysin, shiga-like toxin, and exotoxin A (14, 15). Intriguingly, Fur has also been shown to control two small regulatory RNAs (rhyA and rhyB), which in turn regulate the expression of further genes involved in iron metabolism (16, 17). Thus, Fur can indirectly act as an activator by repressing these small RNAs. On the other hand, Zur is a zinc uptake regulator and acts exclusively as a repressor (10, 18). In the last few years, a number of genes responsible for Zn(II) uptake have been characterized in *E. coli*. In particular, the *znuABC* operon, encoding for a specific triplet of proteins, *ZnuA* (periplasmic protein), *ZnuB* (membrane protein), and *ZnuC* (ATPase), has been shown to be regulated by Zur. When the level of the metal in the media reaches a critical point, Zur repression of the *znu* gene cluster is lifted, allowing the expression of the proteins involved in the ion influx (18–20).
Today *Mycobacterium tuberculosis* continues to be one of the major killers of the century: every year more than eight million people contract tuberculosis and about 2.5 million patients die (21, 22). In 1998, when the *M. tuberculosis* genome was sequenced, many proteins were assigned by sequence homology to previously existing and well characterized families from other organisms (23). Surprisingly, the genome contained homologues of both major families of iron-responsive repressor proteins but no zinc uptake regulator. Although the global iron-dependent regulator IdeR from *M. tuberculosis* (member of the Diphtheria toxin Repressor family) has been structurally and biochemically well characterized (24–26), far less is known about the two Fur genes that were identified. FurA has been characterized zinc uptake regulator. The protein regulates the catalase-peroxidase KatG and is therefore directly involved in isoniazid resistance and virulence (27). FurB, we present results from a wide range of biochemical and biophysical methods indicating that this protein is the iron-dependent regulator IdeR from *M. tuberculosis* (member of the Diphtheria toxin Repressor family) has been structurally and biochemically well characterized (24–26), far less is known about the two Fur genes that were identified. FurA has been shown to regulate the catalase-peroxidase KatG and is therefore directly involved in isoniazid resistance and virulence (27). FurB on the other hand has been shown to be inducible by zinc, but its exact biological role has not yet been clarified (28). To elucidate the biological function and mechanism of *M. tuberculosis* FurB, we present results from a wide range of biochemical and biophysical methods indicating that this protein is the zinc uptake regulator. Furthermore, in this work we present the crystal structure of FurB in complex with Zn$^{2+}$ at a resolution of 2.7 Å. For the first time these results allow the determination of the activation mechanism and provide the structural basis of metal specificity in the Fur/Zur family.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—The furB gene from the *M. tuberculosis* genome H37Rv was cloned into pETM11 vector using HindIII (New England Biolabs, Frankfurt, Germany) and XhoI (New England Biolabs, Frankfurt, Germany) restriction sites and expressed in *E. coli* BL21*DE3* by overnight induction with 1 mM isopropyl β-D-thiogalactopyranoside (Merck) at 20 °C. Because of the introduced tobacco etch virus cleavage site, there is one additional alanine residue inserted at position 2 compared with the original sequence. The protein was purified through nickel-nitrilotriacetic acid affinity chromatography (Qiagen) followed by cleavage of the His$_6$ tag with tobacco etch virus protease and size exclusion chromatography on a Superdex 75 16/60 column (Amersham Biosciences). Dynamic light scattering and mass spectroscopy were performed to confirm the purity of the sample. The concentration was monitored using either a Bradford assay (Bio-Rad) or by measuring the absorption at 280 nm in 6.0 M guanidinium chloride and 0.02 M phosphate buffer, pH 6.5, and using the calculated absorption coefficient (obtained on line from the “Expasy protein chart”).

**Sequence Analyses**—At present *E. coli* Zur is the best characterized zinc uptake regulator. The protein regulates the znu gene cluster encoding for the zinc import system ZnuABC. Sequence searches against the genome were executed with either BLAST (29) or FASTA (30).

**DNA Binding Assays**—Electrophoretic mobility shift assays were performed using canonical Fur-box and a 27-bp almost palindromic DNA sequence identical to the promoter region of Rv2059. The duplex oligonucleotides were mixed with the protein in the presence or absence of different metals in a low salt buffer (10 mM Tris, pH 7.5, 100 mM NaCl, 10% glycerol). The solution was then loaded on a 4–20% native gel, stained with ethidium bromide, and visualized under a UV lamp.

**Micro-PIXE Sample Preparation**—Two samples (the protein as isolated and previously treated with chelating agents) were left for overnight dialysis in 10 mM Tris, pH 8.0, 150 mM NaBr, 10% glycerol, and 5 mM ascorbate. The buffer exchange is necessary to remove any traces of sulfur and chloride. For micro-PIXE measurements of proteins sulfur acts as an internal stand-

| Sample                  | Zn$^{2+}$ | Fe$^{2+}$ | Cu$^{2+}$ |
|-------------------------|-----------|-----------|-----------|
| FurB                    | 0.81 (0.16) | 0.1 (0.05) | 0.28 (0.12) |
| FurB (EDTA)             | 1.15 (0.35) | Bld$^a$   | 0.65 (0.21) |

$^a$ The relatively large errors quoted are due to low counting statistics. The presence of copper is due to contamination during the purification protocol.

$^b$ Bld indicates below the limit of detection.

**Figure 1**. a, electrophoretic mobility shift assay of FurB binding to the promotor region of Rv2059 (lanes 1–3) and the canonical Fur-box (lanes 4–6). b, electrophoretic mobility shift assay of FurB binding to the oligonucleotide of the Rv2059 promotor region with different divalent transition metals.

| Sample                  | Zn$^{2+}$ | Fe$^{2+}$ | Cu$^{2+}$ |
|-------------------------|-----------|-----------|-----------|
| FurB                    | 0.81 (0.16) | 0.1 (0.05) | 0.28 (0.12) |
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**Crystal Structure of M. tuberculosis FurB**

**Table 1**

**Micro-PIXE analysis of FurB in solution as isolated and treated with EDTA**

In each cell the number of metal ions per monomer is reported.

| Sample                  | Zn$^{2+}$ | Fe$^{2+}$ | Cu$^{2+}$ |
|-------------------------|-----------|-----------|-----------|
| FurB                    | 0.81 (0.16) | 0.1 (0.05) | 0.28 (0.12) |
| FurB (EDTA)             | 1.15 (0.35) | Bld$^a$   | 0.65 (0.21) |

$^a$ The relatively large errors quoted are due to low counting statistics. The presence of copper is due to contamination during the purification protocol.

$^b$ Bld indicates below the limit of detection.
ard, and because of the proximity of the x-ray emission energies of sulfur and chlorine, strong chloride fluorescence can affect the accuracy with which the sulfur peak can be quantified (31).

The measurements were carried out at the National Ion Beam Centre, University of Surrey, UK (32). A 2.5-MeV proton beam of \( 2.5 \) m in diameter was used to induce characteristic x-ray emission from the dried liquid droplet (volume \( 0.1 \) l) under vacuum. The x-rays were detected in a solid state lithium-drifted silicon detector with high energy resolution. By scanning the proton beam in \( x \) and \( y \) over the droplet, spatial maps were obtained of all elements heavier than neon present in the sample. Quantitative information was obtained by collecting 3 or 4 point spectra from the sample area. These spectra were analyzed with GUPIX within DAN32 to extract the relative amount of each element of interest in the sample (31).

Crystal Structure Determination—Crystals were obtained by hanging-drop vapor diffusion by mixing the protein at a concentration of 13 mg/ml with the reservoir solution containing 14% glycerol and 0.3 M \((NH_4)_2HPO_4\). Microcrystals with a maximum dimension of 20 \( \mu \)m were initially tested using the micro-focus beam line X06SA at the Swiss Light Source. Most of these crystals did not show any diffraction; however, very few crystals diffracted up to 3.5 Å resolution. Further optimization of purification and crystallization resulted in larger crystals (\( 10 \times 10 \times 40 \) \( \mu \)m), which in favorable cases diffracted to better than 3 Å resolution Tetragonal crystals with \( a = b = 51.6, c = 133.4 \) Å, space group \( P4_2_2_2 \) were mounted directly from the mother liquor and flash-cooled in a cold nitrogen stream (100 K). Multiple anomalous diffraction data across the Zn edge were collected at 100 K at beamline X06SA (Swiss Light Source) using a MarCCD 225 detector. Diffraction data were processed with XDS (33). Three zinc sites per monomer were located using SHELXD (34). Phases from these sites were calculated, extended to 2.7 Å, and refined by solvent flattening as implemented in SHELXE (35). The resulting electron density was readily interpretable (see Figs. 3–5). The refinement against the 2.7 Å native data set collected with a second crystal was compared to SHELX97 (36), DIFFA (37), and "paradise" v. 3.1 (38). The resulting electron density was readily interpretable (39).

![Ribbon diagram of the M. tuberculosis FurB monomer with secondary structural elements annotated.](http://www.jbc.org/) The metal sites are shown as yellow spheres. The DNA binding domain is shown with colors changing from the N terminus in blue to green, and the dimerization domain from yellow to the C terminus in red.

### Crystal Structure Data and Refinement Statistics

| Source | Inflection | Peak | Remote | SAD |
|--------|------------|------|--------|-----|
| Wavelength (Å) | 1.28441 | 1.28358 | 1.26924 | 1.28358 |
| Resolution (Å) | 39.3 (3.2-3.1) | 39.3 (3.2-3.1) | 39.3 (3.2-3.1) | 33.9-2.7 (2.85-2.7) |
| No. of reflections | 52,518 | 52,381 | 52,163 | 89,636 |
| No. of unique reflections | 6138 | 6130 | 6115 | 9342 |
| Redundancy | 5.55 | 5.53 | 5.53 | 9.59 |
| Completeness (%) | 98.3 (100) | 98.4 (100) | 98.4 (100) | 97.8 (92.8) |
| \( \bar{L}/(\sigma) \) | 16.6 (4.3) | 18.9 (6.3) | 18.4 (5.4) | 17.1 (3.2) |
| \( R_{	ext{exp}} \) | 0.12 (0.54) | 0.10 (0.38) | 0.10 (0.43) | 0.097 (0.62) |

* The values in parentheses correspond to the outermost resolution shell.
* Friedel pairs were treated as different reflections.
* \( R_{	ext{exp}} = \sum_{k} \sum_{j} |I(hk\bar{z}) - \bar{I}(hk\bar{z})|/\sum_{k} \sum_{j} |I(hk\bar{z})| \), where \( I(hk\bar{z}) \) is the \( j \)th measurement of the intensity of the unique reflection \( (hk\bar{z}) \), and \( \bar{I}(hk\bar{z}) \) is mean overall symmetry-related measurements.
* r.m.s.d. indicates root mean square deviation.
Crystal Structure of M. tuberculosis FurB

RESULTS

Protein Function and Characterization—M. tuberculosis FurB was purified as a dimer with a molecular mass of 14,596 Da per monomer (data not shown). Micro-PIXE analysis was performed to determine the type and the number of metal ions in the protein. The analysis shows that M. tuberculosis FurB as isolated after the purification as well as after overnight treatment with EDTA contained approximately 1 zinc atom per monomer (Table 1). Surprisingly, it was not possible to complex the protein with Fe$^{2+}$, the natural cofactor of all ferric uptake regulators.

Electrophoretic mobility shift assays were performed to clarify the biological role of FurB. As shown in Fig. 1 the protein does not bind to the canonical Fur-box. Taking into account the strong Zn binding, we identified possible DNA targets by locating a gene cluster in the M. tuberculosis genome that shows similarity to the Zur-regulated znu-ABC operon in E. coli. In the M. tuberculosis genome a triplet of genes, Rv2059 (2,315,172–2,316,707), Rv2060 (2,316,277–2316678), Rv2061c (2,316,679–2,317,083), coding for a hypothetical adhesion protein, a possible conserved integral membrane protein, and a phosphatase, was found that shows high sequence similarity with the E. coli znuABC operon. In the promoter region of this operon an almost perfectly palindromic 27-bp sequence was identified and consequently used for the gel shifts (Fig. 1b). The assays showed that M. tuberculosis FurB binds exclusively to this operator sequence and in addition that only Zn$^{2+}$ can activate the regulator (Fig. 1b). M. tubercu-
Metal-binding Sites of M. tuberculosis FurB in the Crystal—

The crystal structure of FurB possesses three distinct zinc sites (Zn1–Zn3). Two of the metal ions are in the dimerization domain, and one is located in the hinge region between the dimerization and the DNA binding domain. Each of the metal sites exhibits different structural and chemical environments depending on its functional role. Site 1 is surrounded by Asp-62, Cys-76, His-81, and His-83 (Fig. 3). This site occupies a strategic position in the protein structure because it is coordinated by two amino acids from the DNA binding domain (Asp-62 and Cys-76) and two from the dimerization domain (His-81 and His-83). Site 2 is tetrahedrally coordinated to a cluster of sulfur ligands as follows: Cys-86 and Cys-89 from the dimerization domain and Cys-126 and Cys-129 located at the C terminus (Fig. 4). This tetrahedral geometry has already been observed in many other zinc proteins, and the geometrical parameters agree with those previously reported in the literature (43). Site 3 is located in the core of the dimerization domain. Here, the Zn2+ ion is tetrahedrally coordinated by three histidines (His-80, His-82, and His-118) and one glutamate (Glu-101) (Fig. 5). This metal site links the β-sheet of the dimerization domain with the C-terminal α-helix. Because of an initially high B-factor, this zinc site was refined with an occupancy of 0.5.

EXAFS Results of M. tuberculosis FurB—The initial EXAFS experiments in which the protein sample was first dialyzed against an Fe(II)-containing solution and afterward dialyzed against metal-free buffer did not show any significant iron fluorescence. These results further supported the micro-PIXE and gel shift experiments which both indicated no iron binding. The EDTA-treated sample was used to record the zinc-EXAFS to determine the chemical environment of the strongly bound zinc cation. To investigate the nature of the exchangeable metal site(s), a protein sample was incubated with Co2+ ion. The divalent cobalt ion can occupy the site(s) normally reserved for the Zn2+ ion (44). However, the amount of Co2+ in the dialysis buffer had to be kept very low to prevent protein precipitation, and its final concentration was slightly less than the equimolar ratio.

The zinc-EXAFS spectrum and its Fourier transform (FT), characterizing the strongly bound Zn2+, are shown in Fig. 6, a and b. The EXAFS fine structure is dominated by one frequency with intense oscillations at high k. This corresponds to a strong first shell peak at 2.3 Å in the FT typical for metal-sulfur coor-
Crystal Structure of M. tuberculosis FurB

FurB from M. tuberculosis is a DNA-binding protein with a high affinity for Zn\(^{2+}\). The protein is activated by at least one additional Zn\(^{2+}\) cation. It is then able to bind to the promoter region of the znuABC gene cluster encoding proteins that are involved in zinc uptake. The crystal structure presented here thus provides the first view of any member of the Zur family. The overall fold of M. tuberculosis FurB with its N-terminal DNA binding and C-terminal dimerization domain is similar to that of Fur from P. aeruginosa, so far the only available full-length crystal structure of any member of the Fur family (45). The individual domains superimpose well with a root mean square deviation of 1.6 Å for 67 equivalent C-α atoms in the DNA binding domain and 2.0 Å for 50 C-α atoms in the dimerization domain, respectively. The DNA binding fold is also similar to the one from E. coli Fur with a root mean square deviation of 1.6 Å for 68 equivalent residues (46). (All least squares superpositions were calculated using the DALI server (47).) The largest differences between the equivalent domains in each of the crystal structures are at the N terminus where PA Fur possesses an additional helix of 10 residues. The overall structure, however, is very different (Fig. 3). M. tuberculosis FurB adopts a much wider conformation where the two DNA binding domains have moved in a hinge motion of ~77° with respect to the dimerization domain. The hinge region is located between residues 77–79. In addition, there are distinct and important differences in metal binding, which will be discussed in detail below.

Metal Binding and Activation—Three distinct Zn\(^{2+}\)-binding sites were identified in the crystal structure of M. tuberculosis FurB. Zinc 1 is surrounded by two histidines (His-81 and His-83), one cysteine (Cys-76), and one aspartic acid (Asp-62) (Fig. 3, top). The position of this site may be important for the activation of the protein as the metal ion is located at the hinge region between the two domains and may thus be responsible for the orientation of the two domains with respect to each other. This notion is further supported by the cobalt-EXAFS results because the chemical environment corresponds exactly to that determined for Co\(^{2+}\), which presumably occupies the regulatory site. The geometry of this site may also explain the metal selectivity because Zn\(^{2+}\) generally prefers a tetragonal environment with cysteines, whereas Fe\(^{2+}\) is more often found in an octahedral coordination sphere with only nitrogen and oxygen atoms acting as ligands (48, 49). Thus the coordination geometry of Zn1 is likely to be the key parameter for the metal selectivity in the activation of the protein.

Zinc 2 is coordinated tetrahedrally by four cysteines arranged in CXXC sequence motifs (49). The metal ties the C terminus (Cys-126 and Cys-129) to the dimerization domain (via Cys-86 and Cys-89). The same chemical environment is observed in the zinc-EXAFS spectrum. Because Zn\(^{2+}\) can only be removed from this locus by denaturing the protein and tetragonal Zn\(_4\) clusters are by far the preferred motifs for structural zinc sites (43), it is likely that this site serves a structural role.

In the crystal M. tuberculosis FurB contains a third Zn\(^{2+}\) site at

FIGURE 5. Stereoview of the zinc site 3. Top, unbiased experimental electron density after phase extension to 2.7 Å and solvent flattening using SHELXE depicted at 1σ with the final model superimposed is shown. Bottom, final OMIT map where the side chains and the zinc ion were omitted prior to calculating the phases is shown.
lower occupancy, surrounded by three histidines and one glutamate. This site could not be analyzed selectively by absorption spectroscopy, because it was not possible to introduce a third distinguishable metal. Although the exact biological function of this site remains to be determined, there are three possibilities. (i) Metal site 3 could be a second structural site. There are a number of examples reported where a structural zinc site is composed of one aspartate or glutamate and three histidines (43). In this case the location of the metal in the crystal structure of FurB, buried in the dimerization domain coordinating to residues placed in the β-strand S3 and S5, seems to play a stabilization role in the structure. However, in contrast to metal site 1, site 3 is not necessary for structural integrity as it can easily be removed by EDTA treatment. (ii) The site could be an artifact of crystallization. There are a number of cases where additional metal sites have been found in crystal structures; sometimes they are responsible for crystal contacts as in the case of the structure of PA Fur (45). It is remarkable, however, that this site superimposes well with the putative regulatory iron-binding site of the PA Fur crystal structure. After least squares superposition of the dimerization domain (C-α atoms only), the position of the two metals differs by ~1 Å. In addition, three of the four residues binding the metal are identical in PA Fur and M. tuberculosis FurB. However, in the case of PA Fur, this site constitutes an octahedral ligation sphere, well suited for Fe²⁺, whereas in the structure reported here the site forms a tetrahedral environment preferred by Zn²⁺. (iii) It is therefore also possible that site 3 also has a regulatory role so that all three sites have to be filled to constitute an active repressor. Further experiments are clearly needed to elucidate the exact role of this Zn²⁺-binding site.

The EXAFS results reported here are particularly interesting if compared with the x-ray absorption spectroscopy analysis of E. coli Zur metal sites (44) where the authors were also able to add one Co²⁺ ion to the protein already complexed with a very tightly bound zinc ion. For both cations, Zn²⁺ and Co²⁺, respectively, similar chemical environments were identified for the putative structural and regulatory sites in the E. coli Zur (44). For the structural zinc site, a 3 sulfur + 1 nitrogen/oxygen environment was proposed. In this case the difference from the structural ZnS₄ site identified here could be explained either by assuming an average over two sites or by the sequence differences between E. coli Zur and M. tuberculosis Fur. Although the four cysteines coordinating this site are highly conserved, it may be possible that the metal environment is slightly different. In E. coli Zur there is a histidine located close to the N-terminal CXXC motif that could coordinate the Zn²⁺. The chemical environment proposed for the regulatory site occupied by Co²⁺ in E. coli Zur is identical to metal site 1 in our crystal structure (44). However, the residues of the putative regulatory site 1 in the M. tuberculosis FurB crystal structure are not conserved between FurB and E. coli Zur. This comparison further supports the notion that the metal-binding sites have evolved in different directions within the Zur/Fur family.
Although the crystal structure of *M. tuberculosis* FurB presented here shows a detailed picture of the architecture and of the metal-binding sites, the mode of DNA recognition is not immediately obvious. The dimer forms a wide open structure where the two putative DNA recognition helices (H3 and H3′, respectively) are oriented almost parallel to each other. Hence, the binding of each of the DNA recognition elements in the major groove of one half-site of the (pseudo-)palindromic DNA target sequence, as observed in the case of a number of prokaryotic DNA-binding proteins (13), cannot be accomplished without major conformational changes in the hinge region of the protein. Such large conformational changes of the DNA binding domains with respect to the effector binding domains have been reported in the crystal structure of the carbon monoxide oxidation activator protein (CooA) (50). The biological significance of this motion, however, remains controversial as small angle solution scattering experiments indicated that this motion may occur because of crystal packing effects (51).

The electrostatic surface potential shows that helices H1 and H3 possess a mainly positively charged surface. This may be an indication that H1 and its symmetry mate H1′ are also involved in DNA backbone binding. It remains to be seen how sequence specificity is accomplished. The promoter region of *Rv2059* is similar to the promoter of *znuA* and to the Zur-boxes from other organisms; all sequences are pseudo-palindromic and rich in AT base pairs. In the *M. tuberculosis* genome, this is not common as more than 66% of its base pairs are constituted by CG (23). The presence of such a particular sequence of nucleotides, regularly present in the promoter region of a specific class of genes, cannot be regarded just as a mere coincidence and may be an important feature for the recognition and regulation of genes involved in zinc homeostasis. Clearly, a crystal structure of a Fur-Zur DNA complex is needed to unravel the exact mode of sequence-specific DNA binding.

In summary, *M. tuberculosis* FurB shows many structural and functional similarities to *E. coli* Zur. The two proteins share a sequence identity of 28%, and it is likely that the structural zinc site is conserved. *In vivo* the regulator presumably binds the promoter region of a triplet of genes involved in zinc transport. Furthermore, *M. tuberculosis* FurB does not show any iron binding affinity, and the ferric uptake regulator of *M. tuberculosis* has already been identified as FurA. The crystal structure of *M. tuberculosis* FurB presented here shows a similar fold to the structure of Fur from *P. aeruginosa*, but the arrangement of the DNA binding domains with respect to the core of the protein dimer is vastly different, which may indicate a high flexibility and/or even a different binding mode. The differences in metal sites suggest that these sites have evolved differently within the Zur/Fur family depending on which metal is preferred and which genes are regulated. In summary, the combination of absorption spectroscopy, micro-PIXE, and crystallographic data have allowed the investigation and distinction between the structural and the regulatory zinc sites in *M. tuberculosis* FurB. All experimental results presented here suggest a clear biological function for FurB as a zinc uptake regulator.

Acknowledgments—We thank the National Ion Beam Centre, University of Surrey, UK, for micro-PIXE beam access. We are grateful to M. R. Groves for assistance and G. Stockwell for many valuable discussions and support preparing the manuscript.

Addendum—The function of *M. tuberculosis* FurB as the zinc uptake regulator was recently independently determined by Maciag *et al.* (52).

REFERENCES

1. Berg, J. M., and Shi, Y. (1996) *Science* 271, 1081–1085
2. Hantke, K. (2001) *Curr. Opin. Microbiol.* 4, 172–177
3. Braun, V. (2001) *Int. J. Med. Microbiol.* 291, 67–79
4. Finney, L. A., and O’Halloran, T. V. (2003) *Science* 300, 931–936
5. Nelson, N. (1999) *EMBO J.* 18, 4361–4371
6. Hantke, K. (2005) *Curr. Opin. Microbiol.* 8, 196–202
7. Kreikemeyer, B., McIver, K. S., and Podbielski, A. (2003) *Trends Microbiol.* 11, 224–232
8. Gelfand, M. S. (2006) *Curr. Opin. Struct. Biol.* 16, 420–429
9. Stojiljkovic, I., Baumlner, A. J., and Hantke, K. (1994) *J. Mol. Biol.* 236, 531–545
10. Hantke, K. (2001) *Biometals* 14, 239–249
11. Bagg, A., and Neillands, J. B. (1987) *Biochemistry* 26, 5471–5477
12. Diaz-Mireles, E., Wexler, M., Savers, G., Bellini, D., Todd, J. D., and Johnston, A. W. (2004) *Microbiology* 150, 1447–1456
13. Huffman, J. L., and Brennan, R. G. (2002) *Curr. Opin. Struct. Biol.* 12, 98–106
14. Litwin, C. M., and Calderwood, S. B. (1993) *Clin. Microbiol. Rev.* 6, 137–149
15. Vasil, M. L., and Ochsen, U. A. (1999) *Mol. Microbiol.* 34, 399–413
16. Masse, E., and Gottesman, S. (2002) *Proc. Natl. Acad. Sci. U. S. A.* 99, 4620–4625
17. Wilderman, P. J., Sowa, N. A., FitzGerald, D. J., FitzGerald, P. C., Gottesman, S., Ochsen, U. A., and Vasil, M. L. (2004) *Proc. Natl. Acad. Sci. U. S. A.* 101, 9792–9797
18. Gaballa, A., and Helmann, J. D. (1998) *J. Bacteriol.* 180, 5815–5821
19. Patzer, S. I., and Hantke, K. (2000) *J. Biol. Chem.* 275, 24321–24332
20. Patzer, S. I., and Hantke, K. (2001) *J. Bacteriol.* 183, 4806–4813
21. Zignol, M., Hosseini, M. S., Wright, A., Weezenbeek, C. L., Nunn, P., Watt, C. I., Williams, B. G., and Dye, C. (2006) *J. Infect. Dis.* 194, 479–485
22. Kaufmann, S. H. (2006) *Immunity* 24, 351–357
23. Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Ewigleben, K., Gas, S., Barry, C. E., III, Tekaia, F., Badcock, K., Basham, D., Brown, D., Chillingworth, T., Connor, R., Davies, R., Devlin, K., Feltwell, T., Gish, W., Hance, D., Holroyd, S., Hornsey, T., Jones, K., Krog, A., McLean, J., Moule, S., Murphy, L., Ochsenreiter, G., Osborne, J., Quail, M. A., Rajandream, M. A., Rogers, J., Rutter, S., Seder, K., Skelton, I., Squires, R., Squires, S., Squares, R., Squares, S., Statham, A. W., Tekaia, F., Whitehead, S., and Barrell, B. G. (1998) *Nature* 393, 537–544
24. Pohl, E., Holmes, R. K., and Hol, W. G. (1999) *J. Mol. Biol.* 285, 1145–1156
25. Rodriguez, G. M., and Smith, I. (2003) *Mol. Microbiol.* 47, 1485–1494
26. Wisedchaisri, G., Holmes, R. K., and Hol, W. G. (2004) *J. Mol. Biol.* 342, 1155–1169
27. Pym, A. S., Domenech, P., Honore, N., Song, J., Deretic, V., and Cole, S. T. (2001) *Mol. Microbiol.* 40, 879–889
28. Canneva, F., Branzoni, M., Riccardi, G., Provvedi, R., and Milano, A. (2005) *J. Bacteriol.* 187, 5837–5840
29. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* 215, 403–410
30. Lipman, D. J., and Pearson, W. R. (1985) *Science* 227, 1435–1441
31. Grime, G. W., Dawson, M., Marsh, M., McArthur, I. C., and Watt, F. (1991) *Nucl. Instrum. Methods Phys. Res. B* 54, 52–63
32. Kabsch, W. (1993) *J. Appl. Crystallogr.* 26, 797–800
33. Schneider, T. R., and Sheldrick, G. M. (2002) *Acta Crystallogr. Sect. D. Biol.*
Crystal Structure of *M. tuberculosis* FurB

*Molecular Biology of the Cell* 18, 876–880

1. Sheldrick, G. M. (2002) *Z. Kristallogr.* 217, 644–650
2. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) *Acta Crystallogr. Sect. A* 47, 110–119
3. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) *Acta Crystallogr. Sect. D. Biol. Crystallogr.* 54, 905–921
4. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) *Acta Crystallogr. Sect. D Biol. Crystallogr.* 53, 240–255
5. Pettifer, R. F., and Hermes, C. (1985) *J. Appl. Crystallogr.* 18, 404–412
6. Korbas, M., Marsa, D. F., and Meyer-Klaucke, W. (2006) *Rev. Sci. Instrum.* 77, 063105
7. Binsted, N., Strange, R. W., and Hasnain, S. S. (1992) *Biochemistry* 31, 12117–12125
8. Mills, S. A., and Marletta, M. A. (2005) *Biochemistry* 44, 13553–13559
9. Auld, D. S. (2001) *Biomacromolecules* 14, 271–313
10. Outten, C. E., Tobin, D. A., Penner-Hahn, J. E., and O’Halloran, T. V. (2001) *Biochemistry* 40, 10417–10423
11. Pohl, E., Haller, J. C., Mijovilovich, A., Meyer-Klaucke, W., Garman, E., and Vasil, M. L. (2003) *Mol. Microbiol.* 47, 903–915
12. Pecqueur, L., D’Autreux, B., Dupuy, J., Nicolet, Y., Jacquemet, L., Brutscher, B., Michaud-Soret, I., and Bersch, B. (2006) *J. Biol. Chem.* 281, 21286–21295
13. Holm, L., and Sander, C. (1993) *J. Mol. Biol.* 233, 123–138
14. Harding, M. M. (2001) *Acta Crystallogr. Sect. D. Biol. Crystallogr.* 57, 401–411
15. Harding, M. M. (2004) *Acta Crystallogr. Sect. D. Biol. Crystallogr.* 60, 849–859
16. Lanzilotta, W. N., Schuller, D. J., Thorsteinssson, M. V., Kerby, R. L., Roberts, G. P., and Poulos, T. L. (2000) *Nat. Struct. Biol.* 7, 876–880
17. Akiyama, S., Fujisawa, T., Ishimori, K., Morishima, I., and Aono, S. (2004) *J. Mol. Biol.* 341, 651–668
18. Maciag, A., Dainese, E., Rodriguez, G. M., Milano, A., Provvedi, R., Pasca, M. R., Smith, L., Pala, G., Riccardi, G., and Manganelli, R. (2007) *J. Bacteriol.* 189, 730–740
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*J. Biol. Chem.* 2007, 282:9914-9922.
doi: 10.1074/jbc.M609974200 originally published online January 9, 2007

Access the most updated version of this article at doi: 10.1074/jbc.M609974200

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