INFECTION AND IMMUNITY, Nov. 1995, p. 4284–4289 Vol. 63, No. 11
0019-9567/95/$04.00+0

Characterization of an Iron-Dependent Regulatory Protein (IdeR) of *Mycobacterium tuberculosis* as a Functional Homolog of the Diphtheria Toxin Repressor (DtxR) from *Corynebacterium diphtheriae*

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Received 25 April 1995/Returned for modification 9 June 1995/Accepted 11 August 1995

The DtxR protein from *Corynebacterium diphtheriae* is an iron-dependent repressor that regulates transcription from the *tox*, IRP1, and IRP2 promoters. A gene from virulent *Mycobacterium tuberculosis* H37Rv was recently shown to encode a protein, here designated iron-dependent regulator (IdeR), that is almost 60% homologous to DtxR from *C. diphtheriae*. A 750-bp PCR-derived DNA fragment carrying the *M. tuberculosis ideR* allele was subcloned to both high- and low-copy-number vectors. In *Escherichia coli*, transcription from the *C. diphtheriae tox*, IRP1, and IRP2 promoters was strongly repressed by *ideR* under high-iron conditions, and *ideR* restored normal iron-dependent expression of the corynebacterial siderophore in the *C. diphtheriae dtxR* mutant C7(β)hm723. The *M. tuberculosis* IdeR protein was overexpressed in *E. coli* and purified to near homogeneity by nickel affinity chromatography. Gel mobility shift experiments revealed that IdeR bound to a DNA fragment that carried the *C. diphtheriae tox* promoter/operator sequence. DNase I footprint analysis demonstrated that IdeR, in the presence of Cd^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, or Zn^{2+}, protected an approximately 30-bp region on DNA fragments carrying the *tox*, IRP1, or IRP2 promoter/operator sequences. IdeR reacted very weakly in Western blots (immunoblots) with antiserum against the *C. diphtheriae* DtxR protein, suggesting that the immunodominant epitopes of DtxR may be located in its poorly conserved carboxyl-terminal domain.

Iron is an essential nutrient for most living organisms, including many virulent bacterial species. The ability to acquire iron during an infection is an important virulence attribute for a large number of bacterial pathogens (15). In humans and other mammals the amount of iron in body fluids that is available to an invading pathogen is often too low to permit a productive infection. Most extracellular iron is sequestered by heme or heme proteins and by transferrin and lactoferrin. Similarly, most intracellular iron is bound by proteins associated with cellular metabolism or iron storage. Some virulent species of bacteria utilize the low-iron environment of the host as a signal to induce or derepress virulence factors, which include high-affinity iron acquisition systems such as siderophores or membrane-associated proteins involved in obtaining iron directly from heme, transferrin, or lactoferrin (15). Other virulence factors expressed in the iron-depleted environment of the host include various toxins, such as Shiga-like toxin I from *Escherichia coli* (6), exotoxin A from *Pseudomonas aeruginosa* (2), and diphtheria toxin from *Corynebacterium diphtheriae* (19, 20).

Many iron-regulated virulence determinants expressed by gram-negative bacteria are regulated by the Fur protein (ferric uptake regulator) (11, 12, 15). However, in the gram-positive bacterium *C. diphtheriae*, the *tox* gene, which is the structural gene for diphtheria toxin, is regulated by the diphtheria toxin repressor protein DtxR (4, 22, 33). Although Fur and DtxR are both iron-dependent repressors, they share little if any significant amino acid sequence homology (4, 22).

In *E. coli*, the *tox* promoter is constitutively expressed regardless of the iron concentration (4, 22). However, in the presence of the cloned *dtxR* gene, expression from the *tox* promoter in *E. coli* is regulated by an iron-dependent mechanism (4, 22). In the *C. diphtheriae* dtxR mutant C7(β)hm723, production of the corynebacterial siderophore and diphtheria toxin is derepressed in high-iron conditions (3, 14, 22, 30). However, the presence of the cloned wild-type *dtxR* gene in C7(β)hm723 results in the iron-dependent repression of both siderophore and diphtheria toxin syntheses (22). The DtxR protein was purified and shown by DNase I footprinting to bind to an approximately 30-bp region upstream of the *tox* gene (26, 31). The DtxR binding site contains a region of dyad symmetry and overlaps the −10 sequence of the *tox* promoter. The transition metals Cd^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, and Zn^{2+} activate the sequence-specific binding of DtxR to the *tox* operator in vitro (24, 31). Hydroxyl radical footprinting suggested that DtxR binds in a symmetrical manner about the dyad axis of the *tox* operator and that DtxR binds to DNA as a dimer or multimer (24).

Two additional DtxR and iron-regulated promoter/operator sequences, designated IRP1 and IRP2, were recently cloned from the *C. diphtheriae* chromosome (25). Gel shift and DNase I footprinting experiments indicated that DtxR binds to DNA fragments carrying the IRP1 and IRP2 promoters and that the binding is metal dependent. A 19-bp consensus DtxR binding site was derived from a comparison of the various DtxR operator sequences (25) and by in vitro affinity selection (32). The

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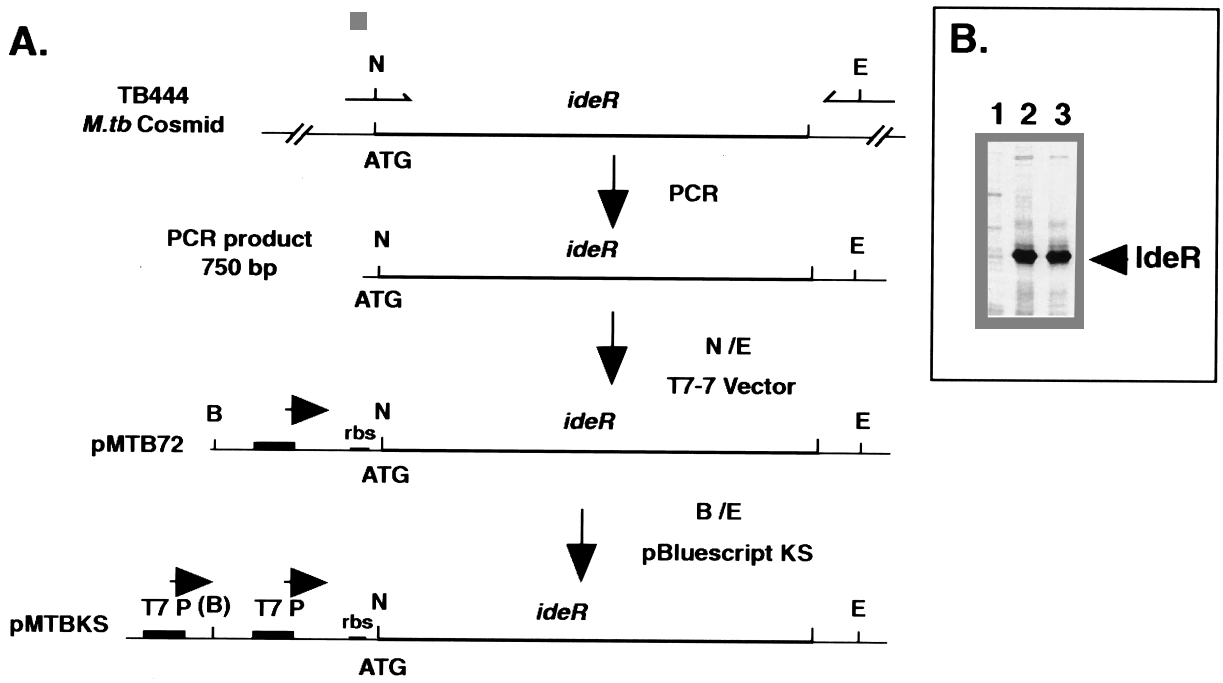

FIG. 1. (A) Strategy used to subclone the *ideR* gene of *M. tuberculosis* from cosmid TB444 into cloning/expression vectors pT7-7 and pBluescript KS (for detailed information see Materials and Methods). Only the *ideR* gene and relevant flanking regions and restriction sites are shown (not to scale). Restriction sites: N, *Nde*I; E, *Eco*RI; B, *Bgl*II; (B), *Bgl*II site ligated to *Bam*HI site in pBluescript; neither restriction site is regenerated. Arrows located above the TB444 map indicate primers used for PCR: primer TB1 contains an *Nde*I site (N), and primer TB2 contains an *Eco*RI site (E). T7P indicates the phage T7 gene 10 promoter, and arrows indicate the direction of transcription. rbs, T7 gene 10 ribosome binding site; ATG, start codon for *ideR*. (B) Expression of IdeR protein using T7 expression system and analysis of bacterial extracts by SDS-PAGE. Proteins were labeled in vivo with [^{35}S]methionine in *E. coli* DH5α/pGP1-2 carrying the pT7-7 vector (lane 1), pMTB72 (lane 2), or pMTBKS (lane 3).

consensus DtxR binding site contains a perfect 9-bp inverted repeat interrupted by a single base pair. The sequence of the 19-bp consensus DtxR binding site has little if any similarity to the consensus Fur binding site from *E. coli* (15). This lack of homology is consistent with the inability of DtxR to bind to a Fur operator sequence (26). Additionally, it was shown that *dtxR* has no effect on the expression of several Fur-regulated proteins in *E. coli* (4). Likewise, transcription from the DtxR-regulated *tox*, IRP1, and IRP2 promoters is not regulated by *fur* (4, 22, 25). The recent finding of a dtxR homolog in *Brevibacterium lactofermentum* (18), a coryneform bacterium closely related to *C. diphtheriae*, along with the identification of DtxR-like binding sites upstream of iron-regulated genes in *Streptomyces pilosus* (10) and *Mycobacterium smegmatis* (9), suggest that repressors homologous to DtxR are present in other gram-positive bacteria.

The cloning and nucleotide sequence of *dtxR* homologs from *Mycobacterium tuberculosis* and several other mycobacteria are described elsewhere (7). Here we propose *ideR* as an appropriate symbol for genes that encode iron-dependent regulatory proteins homologous with *dtxR* in mycobacteria or other bacteria that do not make diphtheria toxin. In this study, we subcloned the *M. tuberculosis ideR* allele and showed that it is a functional homolog of *dtxR* that represses transcription of *C. diphtheriae* iron-regulated promoters. Additionally, we purified the *M. tuberculosis* IdeR protein and demonstrated by DNase I footprinting that it binds to the *C. diphtheriae tox*, IRP1, and IRP2 operator sequences in the presence of divalent metals.

MATERIALS AND METHODS

DNA manipulation. The PCR was used to amplify the *ideR* gene from cosmid TB444 (kindly supplied to I. Smith by Stewart Cole), using two oligonucleotide primers (TB1, 5′-ATGGAGGGTGC**CATATG**AACGAGTTG-3′; TB2, 5′-AAC AACTCG**GAATTC**GACTGCCGC-3′). These primers introduced unique restriction sites, indicated in bold type, at either the 5′ (*Nde*I, TB1) or 3′ (*Eco*RI, TB2) end of the 750-bp PCR-derived DNA fragment carrying *ideR*. The *Nde*I site introduced by primer TB1 at the 5′ end of the PCR product contains an ATG sequence which serves as the start codon for the *ideR* gene. The *Eco*RI site inserted by TB2 is 64 bp downstream from the stop codon for *ideR*. The PCR fragment was digested with *Nde*I-*Eco*RI and ligated into the *Nde*I-*Eco*RI site in the PT7-7 vector, creating plasmid pMTB72 (Fig. 1A). A 1.1-kb *Bgl*II-*Eco*RI fragment from pMTB72 was ligated into the *Bam*HI-*Eco*RI site in the high-copy-number pBluescript KS vector to generate plasmid pMTBKS and into the *Bam*HI-*Eco*RI site in the low-copy-number vector pWKS130 to create plasmid pMTBW130 (Fig. 1A). Since vector pWKS130 contains the multiple cloning site and flanking regions from pBluescript KS, the restriction and genetic map shown for pMTBKS in Fig. 1A is identical to that for pMTBW130. Other plasmids used in this study are listed in Table 1.

The nucleotide sequence of the insert in plasmid pMTB72 was found to contain a single C-to-T nucleotide change from that reported by Doukhan et al. (7). This base change, which is located 93 bp from the 5′ end of the gene, did not change the predicted amino acid sequence for the recombinant IdeR protein encoded by pMTB72.

Expression and purification of the *M. tuberculosis* IdeR protein. IdeR was overexpressed in *E. coli* DH5α carrying plasmids pMTBKS and pGP1-2, using the T7 expression system as described previously (24, 29). Ten-milliliter cultures were sonicated, and IdeR present in the bacterial extract was purified by nickel affinity chromatography using nickel-activated NTA-agarose (Qiagen Inc., Chatsworth, Calif.). Greater than 90% of the IdeR protein in the bacterial extract bound to the column (data not shown), and bound protein was eluted using a step gradient of histidine as described previously (24). Approximately 500 μg of IdeR was obtained, and peak protein fractions were eluted from the column at 20 to 30 mM histidine. The protein was at least 90% pure as determined by Coomassie blue staining of proteins after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

β-Galactosidase assays. Bacterial cultures were grown overnight in LB medium supplemented with appropriate antibiotics to maintain plasmids (16). LB medium contained either 500 μg of ethylenediamine-di-*o*-hydroxyphenylacetic acid (EDDA) per ml (−Fe) or no added EDDA (+Fe). Bacterial cultures were processed and measurements of β-galactosidase activity were determined as described previously (17, 25).

TABLE 1. Plasmids used in this study

Plasmid	Relevant property(ies)	Source or reference
pQF50	Promoter/probe vector	8
pQFtox	*tox-lacZ* fusion in pQF50	25
pIRP1-1	IRP1-*lacZ* fusion in pQF50	25
pIRP2	IRP2-*lacZ* fusion in pQF50	25
pDSK29	*dtxR*$^+$	23
pGP1-2	Temperature-inducible T7 RNA polymerase gene	29
pBluescript KS	High-copy-number cloning/expression vector	Stratagene, La Jolla, Calif.
pMTBKS	*ideR*$^+$ in KS	This study
pT7-7	Expression vector	29
pMTB72	*ideR*$^+$ in pT7-7	This study
pWKS130	Low-copy-number vector	34
pMTBW130	*ideR*$^+$ in pWKS130	This study
TB444	*ideR*$^+$ in pYUB18 cosmid	Stewart Cole
pKN2.6	Shuttle vector, Kn^r	22
pMTBKN	*ideR*$^+$ in pKN2.6	This study
pCMS97	*dtxR*$^+$ in pCM2.6	22

Gel mobility shift assays and DNase I footprinting. Gel mobility shift assays and DNase I footprinting were done as described by Schmitt et al. (26). Purified DtxR or IdeR was present at 500 nM, and divalent metals were used at 150 μM in all DNA binding experiments, unless indicated. DNA fragments carrying the various promoter/operator sequences were end labeled at their 3′ end with ^{32}P using the Klenow fragment as described previously (16, 26).

Siderophore assays. *C. diphtheriae* strains harboring various plasmids and supplemented with kanamycin (25 μg/ml) or chloramphenicol (2 μg/ml) were grown overnight in modified PGT medium (30) that was treated with Chelex-100 (Bio-Rad Laboratories, Richmond, Calif.). Modified PGT medium contained either 10 μM $FeSo_4$ (+Fe) or no added iron (−Fe). The chrome azurol S assay procedure (27) as modified by Tai et al. (30) was used to quantitate siderophore levels present in culture supernatants. Siderophore units were determined as described previously (30).

Immunoblotting. Twofold serial dilutions of nondenatured, purified DtxR or IdeR were applied to nitrocellulose with a dot blot apparatus. Five micrograms of protein was present in the initial undiluted sample. The immobilized protein samples were incubated with rabbit antiserum raised against a MalE-DtxR fusion protein (26) and then reacted with a peroxidase-conjugated anti-rabbit immunoglobulin G. A chromogenic substrate for peroxidase was used to develop the blot as described previously (13).

RESULTS

Subcloning and expression of *ideR* from *M. tuberculosis*. The *ideR* gene on cosmid TB444 was subcloned onto the expression vectors pT7-7 and pBluescript KS to generate plasmids pMTB72 and pMTBKS, respectively (see Materials and Methods and Fig. 1A). The T7 expression system (29) was used to preferentially express the cloned *ideR* gene present on plasmids pMTB72 and pMTBKS in DH5α carrying plasmid pGP1-2. Expressed proteins were labeled with [^{35}S]methionine, and bacterial extracts were subjected to SDS-PAGE (Fig. 1B). The IdeR protein was strongly expressed from both pMTB72 and pMTBKS, and no protein corresponding to IdeR was detected in the pT7-7 vector control.

Regulation of *C. diphtheriae* promoters by IdeR. In a recent study, we demonstrated that transcription from the *C. diphtheriae tox*, IRP1, and IRP2 promoters, on plasmids pQFtox, pIRP1-1, and pIRP2, respectively, was iron regulated in *E. coli* only in the presence of the cloned *C. diphtheriae dtxR* gene (25). All three promoters were derepressed in the absence of *dtxR* regardless of the iron concentration. The results of β-galactosidase assays shown in Table 2 indicate that the cloned *ideR* gene on the low-copy-number plasmid pMTBW130 fully repressed transcription of the *tox*, IRP1, and IRP2 promoters under high-iron conditions. The three *C. diphtheriae* promoter sequences are present as operon fusions to a promoterless *lacZ* gene in the promoter-probe vector pQF50 (8, 25). The level of repression of the *C. diphtheriae* promoters by the *ideR* gene in high-iron medium was similar to the level of repression observed with the *C. diphtheriae dtxR* gene encoded on plasmid pDSK29 (Table 2) (25). When the *ideR* gene was on a high-copy-number plasmid, such as pMTBKS, significant repression of all three promoters was observed even under low-iron conditions (data not shown). Similar repression under low-iron conditions was also observed when the *C. diphtheriae dtxR* gene was present in high-copy plasmids (23).

TABLE 2. Effect of the cloned *ideR*$^+$ allele on expression of iron-regulated promoters from *C. diphtheriae*

Plasmid(s)[a]	β-Galactosidase (U)[b]	
	+Fe	−Fe
pQF50	1.2 (±0.5)	1.1 (±0.3)
pQFtox	16.2 (±1.3)	18.7 (±3.1)
pQFtox/pDSK29 (*dtxR*$^+$)	<1.0	18.9 (±2.0)
pQFtox/pMTBW130 (*ideR*$^+$)	<1.0	12.3 (±1.1)
pIRP1-1/pMTBW130 (*ideR*$^+$)	<1.0	22.1 (±3.3)
pIRP2/pMTBW130 (*ideR*$^+$)	<1.0	45.1 (±5.5)

[a] All plasmids are present in DH5α.
[b] Units were determined according to Miller (17). Values are averages of three independent experiments ± standard deviations.

Since no mycobacterial DNA sequences 5′ to the ATG start codon were present in the PCR-derived DNA fragment that carried the *ideR* gene, it is assumed that plasmid pMTBW130 does not carry the native *Mycobacterium* promoter for *ideR* (see Materials and Methods). Additionally, since plasmid pGP-1-2, which encodes the T7 RNA polymerase, is not present in any of the strains used in the β-galactosidase experiments (Table 2), the vector-encoded T7 promoter is not active. We presume, therefore, that an endogenous vector promoter is directing transcription of the *ideR* allele in plasmid pMTBW130.

Overexpression and purification of IdeR. The *ideR* gene encoded on plasmid pMTBKS was overexpressed by using the T7 expression system as described previously (24, 29). The IdeR protein synthesized by this method represented a significant portion of the total cellular protein present in the bacterial extract (Fig. 2, lane 2). The IdeR protein in the bacterial extract was purified to near homogeneity by nickel affinity

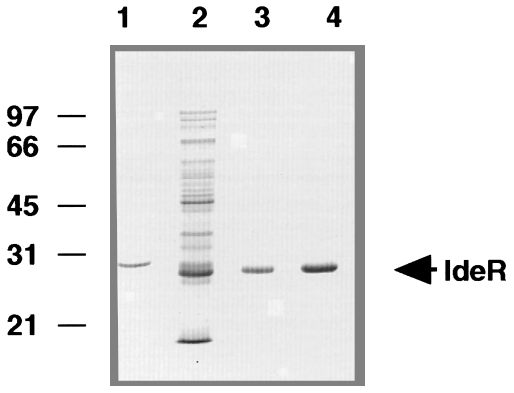

FIG. 2. Purification of the IdeR protein. The IdeR protein was overexpressed from plasmid pMTBKS carried by DH5α/pGP1-2 using the T7 expression system. Proteins were separated by SDS-PAGE and stained with Coomassie blue. Lane 1, DtxR; lane 2, whole-cell extract containing IdeR; lanes 3 and 4, purified fractions of IdeR obtained after histidine elution from a nickel affinity column.

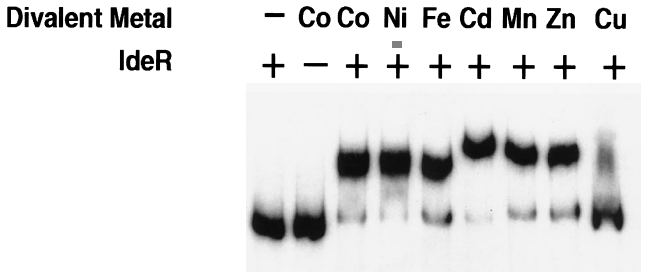

FIG. 3. PAGE shift assays. Purified IdeR was incubated with a ^{32}P-labeled 320-bp DNA fragment that carries the *tox* promoter/operator sequence (24). The IdeR-DNA complex was electrophoresed on a nondenaturing acrylamide gel (5%). The gel was dried and analyzed by autoradiography. Various divalent metals (as indicated) were used to activate the binding activity of IdeR.

chromatography as described in Materials and Methods. Purified fractions obtained after elution with histidine are shown in lanes 3 and 4. The *C. diphtheriae* DtxR protein (lane 1) and the *M. tuberculosis* IdeR protein have slightly different mobilities on SDS-PAGE, although they differ in mass by only 100 Da. The difference in migration is likely due to the slight charge difference that exists between these proteins (7).

Interaction of IdeR with *C. diphtheriae* operators. Gel shift assays revealed that purified IdeR protein altered the electrophoretic mobility of a 320-bp DNA fragment that carries the *C. diphtheriae tox* promoter/operator sequence (Fig. 3). The interaction of IdeR with this DNA fragment occurred only in the presence of divalent metals. DNase I footprinting experiments using the 320-bp *tox* promoter/operator fragment indicated that IdeR protected an approximately 30-bp region from DNase I digestion (Fig. 4). The sequences protected by IdeR at the *tox* operator correspond to the previously identified binding site for the *C. diphtheriae* DtxR protein (Fig. 3) (26). The sequence-specific binding of IdeR was activated by Cd^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, or Zn^{2+}; however, Cu^{2+} did not function as a cofactor for IdeR. Additionally, the IdeR protein was comparable to the *C. diphtheriae* DtxR protein in its capacity to protect from DNase I cleavage the IRP1 and IRP2 promoter/operator sequences from *C. diphtheriae* (Fig. 5).

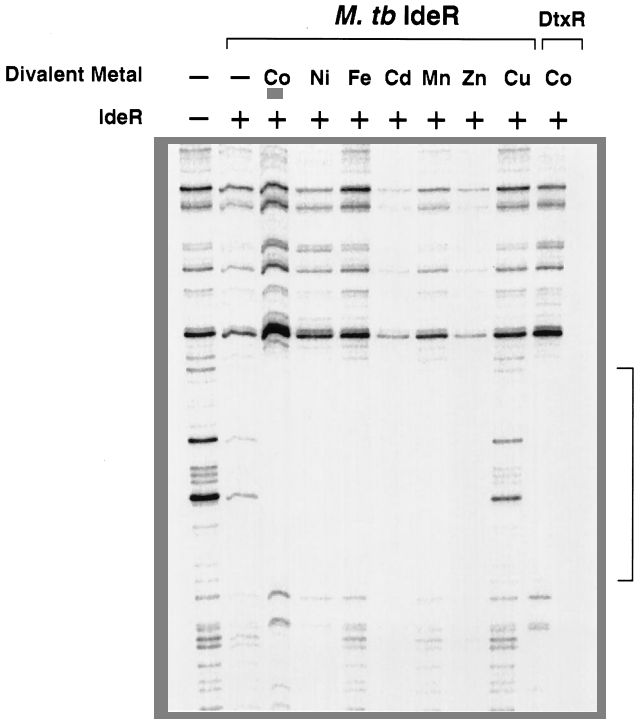

FIG. 4. DNase I footprinting at the *tox* operator. Purified IdeR or DtxR as indicated were analyzed in DNase I footprinting experiments using a 320-bp DNA fragment carrying the *tox* operator sequence. DNase I footprinting was done as described previously (26). The bracket to the right of the gel indicates sequences protected from DNase I digestion.

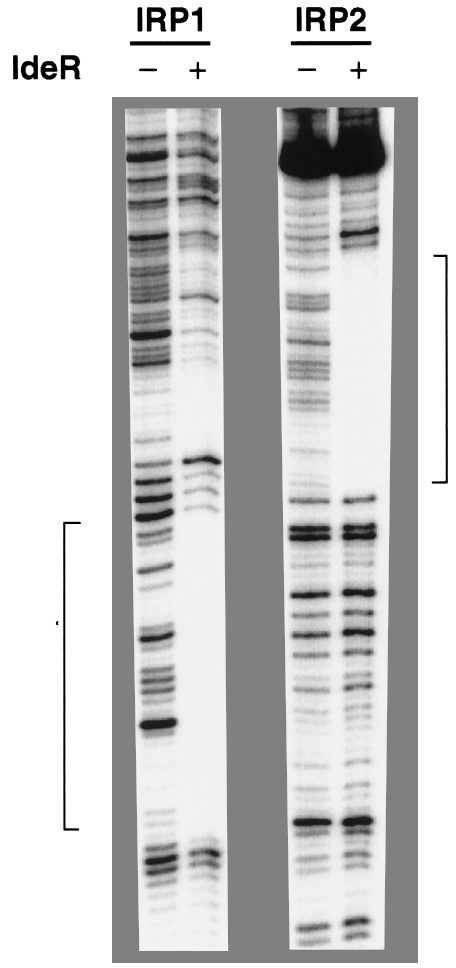

FIG. 5. DNase I footprinting at the IRP1 and IRP2 operators. Purified IdeR was analyzed in DNase I footprinting experiments in the presence of 150 μM Co^{2+} with DNA fragments carrying either the IRP1 promoter/operator (460-bp *Alu*I fragment) or the IRP2 promoter/operator (220-bp *Hae*III DNA fragment). Brackets flanking the footprinting gel indicate DNA sequences protected from DNase I digestion. The bracketed sequences were previously shown to be protected by the *C. diphtheriae* DtxR protein (25).

Regulation of siderophore expression. *C. diphtheriae* C7(β) hm723, which carries a point mutation in the *dtxR* gene (3, 23), produces a defective DtxR repressor protein that fails to fully repress siderophore synthesis in high-iron medium (Table 3) (22). However, the cloned wild-type *dtxR* gene from *C. diphtheriae* in plasmid pCMS97 in C7(β)hm723 represses siderophore production under high-iron conditions to wild-type levels (Table 3) (22). Plasmid pMTBKN, which carries the *ideR* gene from *M. tuberculosis* on the *E. coli-C. diphtheriae* shuttle vector pKN2.6, was transformed by electroporation into both wild-type strain C7β and *dtxR* mutant C7(β)hm723. Although transcription of *C. diphtheriae dtxR* on plasmid pCMS97 is directed from the native *dtxR* promoter (22), *ideR* on plasmid

TABLE 3. Regulation of siderophore synthesis in *C. diphtheriae* by the cloned *ideR*$^+$ allele from *M. tuberculosis*

Strain/plasmid	Siderophore (U[a])	
	+Fe	−Fe
C7(β) (*dtxR*$^+$)	<0.5	72.1 (±7.4)
C7(β)/pMTBKN (*ideR*$^+$)	<0.5	68.9 (±7.9)
C7(β)/pCMS97 (*dtxR*$^+$)	<0.5	83.2 (±3.4)
C7(β)hm723 (*dtxR*)	17.4 (±3.5)	71.8 (±2.0)
C7(β)hm723/pMTBKN (*ideR*$^+$)	<0.5	71.3 (±4.5)
C7(β)hm723/PCMS97 (*dtxR*$^+$)	<0.5	88.9 (±9.7)

[a] Units were determined as described previously (22). Values are averages of three independent experiments ± standard deviations.

pMTBKN is presumably transcribed from promoters present on the shuttle vector pKN2.6, since its native promoter is not present on the cloned insert DNA. As shown in Table 3, the presence of the *ideR* gene in pMTBKN resulted in full repression of siderophore synthesis in high-iron medium in C7(β)hm723. The presence of the shuttle vector or plasmid pMTBKN did not affect the normal iron-regulated expression of the siderophore in the wild-type strain, C7β (Table 3). The results indicate that the IdeR repressor regulates transcription from a *C. diphtheriae* promoter(s) involved in the expression of genes required for the biosynthesis of the corynebacterial siderophore.

Immunoblotting. Polyclonal antiserum raised against the *C. diphtheriae* DtxR protein was tested in dot blot immunoassays with purified DtxR or IdeR. As expected, the *C. diphtheriae* DtxR protein reacted strongly with the antiserum, while the IdeR protein, at concentrations equivalent to those of *C. diphtheriae* DtxR, reacted very weakly with the antiserum, despite the high homology that exists in the amino-terminal 140-amino-acid sequences of DtxR and IdeR (data not shown).

DISCUSSION

In this study, we showed that the *ideR* gene from *M. tuberculosis* regulates transcription from the *C. diphtheriae tox*, IRP1, and IRP2 promoters by an iron-dependent mechanism, using an *E. coli lacZ* reporter system. The IdeR protein was overexpressed and purified by nickel affinity chromatography, and the purified protein bound to three different *C. diphtheriae* DtxR operators in a metal-dependent manner.

In a separate study, Doukhan et al. (7) cloned and determined the nucleotide sequence of the *ideR* gene. The IdeR protein had 59% overall amino acid identity with *C. diphtheriae* DtxR. However, the two proteins are 78% identical over 140 residues at the N terminus and are almost 90% homologous in this region if conserved amino acids are included in the comparison. Although *Mycobacterium* and *Corynebacterium* are related genera, the very high degree of homology at the N terminus between DtxR and IdeR was surprising. In the present study we provide direct experimental evidence that IdeR recognized and interacted with *C. diphtheriae* DtxR-regulated operator sequences both in vitro and in vivo as efficiently as the *C. diphtheriae* DtxR protein.

The crystal structure of the *C. diphtheriae* DtxR protein in complex with divalent cations has recently been determined (21). The three-dimensional structure revealed that DtxR is a dimer and that each monomer of DtxR contains three distinct domains. Domains 1 and 2, which constitute the first 144 amino acids at the N terminus, include residues involved in DNA binding, metal binding, and the formation of the dimer interface. Interestingly, domains 1 and 2 correspond to the sequence that is most highly conserved between DtxR from *C. diphtheriae* and IdeR from *M. tuberculosis*. No function or activity has been established for domain 3, which represents the carboxyl-terminal 40% of the protein. DtxR from *C. diphtheriae* and IdeR from *M. tuberculosis* share only 25% identity in this C-terminal region (7).

Within the last decade the incidence of tuberculosis has increased in the United States, due to a large degree to the significant rise in AIDS cases (28). In nonindustrialized countries throughout the world, tuberculosis continues to be a serious public health problem. It is the leading cause of mortality from a single infectious organism, and it causes the deaths of 2.5 to 3.0 million people annually (28). Although numerous investigators are studying *M. tuberculosis*, progress has been slow because of the long generation time of the bacteria, the paucity of genetic tools, and the poorly understood virulence mechanisms of this organism.

In many gram-negative bacteria, iron-regulated genes are essential for expression of full virulence. It is likely that the acquisition of iron by *M. tuberculosis* is also essential for growth and survival of this important bacterial pathogen during the course of an infection. *M. tuberculosis* synthesizes two distinct iron-regulated siderophores: the cell-associated mycobactin and the excreted siderophore exochelin (35). Additionally, *M. tuberculosis* and other *Mycobacterium* species produce a number of iron-regulated membrane proteins (35). Fiss et al. (9) have recently cloned and sequenced genes involved in the synthesis (*fxbA*) and uptake of ferric exochelin from *M. smegmatis*. Expression of the *fxbA* gene was shown to be iron regulated in *M. smegmatis*. Two separate sequences upstream of the *fxbA* coding region have some homology to the consensus DtxR binding site, but it has not yet been demonstrated directly whether these specific sequences function as DtxR binding sites.

Although the diphtheria toxin repressor gene, *dtxR*, was originally named for its ability to regulate the *tox* gene in *C. diphtheriae*, recent findings (25) indicate that DtxR is a global iron-dependent regulatory factor in *C. diphtheriae* similar to the Fur protein in gram-negative bacteria (1, 5). With the discovery of DtxR homologs in *M. tuberculosis*, several other mycobacterial species, and *B. lactofermentum* (18) and with the high probability that DtxR-like homologs exist in some other gram-positive bacteria, this is an appropriate time to address the issue of nomenclature for the homologs of *dtxR*. Use of the designation *dtxR* (diphtheria toxin repressor) is likely to cause confusion with genes from bacterial species that do not produce diphtheria toxin. We propose, therefore, to introduce a more general designation, *ideR*, for iron-dependent regulators, for the family of genes, represented by *dtxR* and its homologs, that encodes products that function as iron-dependent global regulatory proteins. Because the designation *dtxR* is firmly established and has been widely used in the literature for several years, it remains appropriate as an alternative to *ideR* in *C. diphtheriae* and other corynebacterial species that produce diphtheria toxin.

ACKNOWLEDGMENTS

This research was supported in part by PHS grant AI-14107 from the NIAID awarded to R.K.H. and by PHS grant GM-19693 awarded to I.S.

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Editor: J. R. McGhee