Review

Comparative Genomic Insights Into the Biosynthesis and Regulation of Mycobacterial Siderophores

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Key Words

Mycobacteria • Siderophore • Biosynthesis • Anti-tuberculosis

Abstract

Iron is essential for nearly all biological events. Siderophores are indispensable for most organisms to obtain iron from iron-limiting milieus. This holds particularly true for pathogens such as the causative agent of tuberculosis -- *Mycobacterium tuberculosis*. The categories of mycobacterial siderophores, their biosynthesis and regulation are summarized here. The siderophore biosynthesis and regulation differences between the pathogenic and non-pathogenic mycobacteria are highlighted from comparative genomic perspective, with an aim to find clues for drug or drug target within siderophore metabolism.

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Introduction

Tuberculosis (TB) remains a major public health problem worldwide. According to the 2011 WHO Report [1], there were 8.8 million incidences of tuberculosis globally. The problem is exacerbated by the emergence of multidrug-resistant strains (MDR-TB) and extensively drug-resistant MTB (XDR-TB). New drug or vaccine targets are urgently needed.

Iron is essential for mycobacterial growth *in vitro*, and obtaining this essential element is a formidable challenge to all pathogenic mycobacteria grown *in vivo*. Hence, the ability to obtain iron from the host is critical for pathogen proliferation, pathogenesis and latency. However, host iron is sequestered by transferrins and lactoferrins, heme components, Fe-S

clusters and ferritins [2]. Once infected, the host immediately primes immunoprotection mechanisms to adjust internal iron levels, such as synthesizing hepcidin [3] and siderocalin [4], to fight against pathogens [5, 6]. The mystery of the hitherto most successful pathogen -- *Mycobacterium tuberculosis* (*M. tuberculosis*) -- might be its superb ability to obtain iron via its high affinity siderophores. Evidence for this possibility is the observation that the growth of siderophore-deficient mycobacteria is hampered severely within intracellular iron-limiting milieu [7, 8].

Much research has been performed with mycobacterial siderophores. As early as in 1945, mycobactin was the target of chemotherapy [9]. More recently, many researchers [10-13] have used siderophores as models of antibacterial/antituberculosis drugs, in the hope of obtaining effective anti-TB agents. Some agents developed in these studies do kill *M. tuberculosis* [14], and spurred intensive study.

Categories of mycobacterial siderophores

Siderophores are small molecular chelators produced by most microorganisms and are important for iron uptake and transportation. Siderophores have multiple structures, special receptors and are regulated by environmental iron levels [15]. The survival and pathogenesis of microbes is closely related to their siderophores. For example, pyoverdinin-deficient *Pseudomonas aeruginosa* mutants became avirulent when injected into burned mice [16]. Siderophores are also involved in the microbial response to oxidative stress [17, 18]. In addition, siderophores are essential for *M. smegmatis* biofilm formation during iron starvation [19].

The major mycobacterial siderophores include the extracellular carboxymycobactin and exochelin and the intracellular mycobactin. Carboxymycobactins have a shorter alkyl side chain which terminates in a methyl ester, which renders carboxymycobactins more polar and soluble than mycobactin. This facilitates the secretion of carboxymycobactins into the aqueous extracellular milieu to obtain iron from environmental sources, including the host. Exochelin, a low-molecular-weight polypeptide synthesized through a non-ribosomal pathway, consists of unusual peptide bonds that protect the molecule from peptidase hydrolysis. The three hydroxamate groups of exochelin form the rigid hexahedral Fe³⁺ chelating centre.

The siderophores of the pathogenic *M. tuberculosis* consist of carboxymycobactin and mycobactin [20, 21], while saprophytic *M. smegmatis* produces mycobactin and extracellular carboxymycobactin and exochelin [9, 20, 22-24]. Exochelin MN, isolated from *M. neoaurum* culture broth, is a peptide with unusual β -hydroxyhistidine and *N*-methyl groups. The molecule utilizes the *cis*-hydroxamate motif, the -OH and the imidazole nitrogen of β -OH-His, to coordinate iron [25] and these groups release iron in a pH-dependent manner [26]. Exochelin MN can provide iron for the *M. leprae*, even though this siderophore is not found in this bacterium [27].

Biosynthesis of the mycobacterial siderophores

Mycobactin

Mycobactin is assembled by a series of polyketide synthase/nonribosomal peptide synthetases in *M. tuberculosis* [28]. Two gene clusters, ie, a 24 kb genetic fragment containing the *mbt*-1 gene cluster and the unlinked *mbt*-2 cluster are involved in mycobactin production (Table 1). The genes in the former cluster were designated as *mbtA-J* and mediated the synthesis of the mycobactin core scaffold [28], while genes in the latter cluster were involved in the assembly of mycobactin side chains [29]. The genes in *mbt*-2 cluster were annotated as *mbtK*, *mbtL*, *mbtM* (*fadD33*), and *mbtN* (*fadE14*) for *Rv1347c*, *Rv1344*, *Rv1345*, and *Rv1346*, respectively [29]. A systematic mutational analysis found that *mbtH* is dispensable for the

Table 1. Mycobacterial genes, gene products and the putative functions involved in mycobactin biosynthesis

Gene clusters	Rv. No.	Genes	Gene products	Putative functions
mbt-1	Rv2384	<i>mbtA</i>	MbtA	salicyl-AMP ligase/salicyl-S-ArCP synthetase
	Rv2383c	<i>mbtB</i>	MbtB	peptide synthetase, editing theacyl carrier protein (ArCP)
	Rv2382c	<i>mbtC</i>	MbtC	polyketide synthase
	Rv2381c	<i>mbtD</i>	MbtD	polyketide synthase
	Rv2380c	<i>mbtE</i>	MbtE	peptide synthetase
	Rv2379c	<i>mbtF</i>	MbtF	peptide synthetase
	Rv2378c	<i>mbtG</i>	MbtG	acetyl hydroxylase
	Rv2377c	<i>mbtH</i>	MbtH	stimulate amino acid adenylation steps [71]
	Rv2386c	<i>mbtI</i>	MbtI	Isochorismate synthase/salicylate synthase
	Rv2385	<i>mbtJ</i>	MbtJ	acetyl hydroxylase
mbt-2	Rv1347c	<i>mbtK</i>	MbtK	GCN5 N- acetyltransferase
	Rv1344	<i>mbtL</i>	MbtL	acyl carrier protein
	Rv1345	<i>mbtM</i>	FadD33	acyl- AMP ligase/ acyl-CoA synthase
	Rv1346	<i>mbtN</i>	FadE14	acyl-ACP dehydrogenase [29]

M. smagmetis mycobactin production [30]. The orthologs of *mbtA*, *mbtB*, *mbtC*, *mbtD*, *mbtE*, *mbtF*, *mbtG*, and *mbtT* (*mbtB* and *mbtT* fused into a single gene in the *M. tuberculosis*) present in diverse phylogenetic groups and with similar orientation. Horizontal gene transfer of the whole or a part of the *mbt* gene cluster did occur across large evolutionary families [30].

The reactions involved in the synthesis of mycobactin are shown in Fig. 1. The bifunctional enzyme MbtI mediates a sequential reaction, in which chorismic acid is firstly converted into isochorismate and then into salicylate (the initial precursor of mycobactin) [31, 32]. MbtA, the salicyl-AMP ligase/salicyl-S-ArCP synthetase, activates and transfers the salicylate to the ACP(acyl carrier protein) site of MbtB to form salicyl-S-ACP(I). Then, Ser/Thr is linked into salicyl-S-ACP and converted into a phenyloxazoline ring [28](I). Subsequently, a lysine activated by peptide synthetase MbtE or MbtF(III) is attached to the peptide chain [33, 34]. Then malonyl-CoA and acetyl-CoA are integrated into the lysine residue by polyketone synthase MbtC/D [7, 34](II). Another lysine activated by peptide synthase MbtE or MbtF (III) is added to this peptide chain [34]. When these amino acids are cyclized, the assemblage of core structure of mycobactin is complete (IV). Biosynthesis of the lipid side chain is as follows [29] (V): FadD33, the acetyl-CoA synthase homolog of mycobacterial acyl-AMP ligase [35, 36], activates the lipid molecules required for mycobactin biosynthesis. Acyl-ACP dehydrogenase FadE14 shares high level similarity with acyl-CoA dehydrogenases, which catalyze the dehydrogenation of acyl-CoA thioesters to the related *trans*-2-enoyl-CoA [37, 38], and dehydrogenates the ACP-tethered products and transforms them into α , β -unsaturated lipid chain. Subsequently, the unsaturated lipid chains are directly transferred onto the ϵ -amino group of Z-Lys-OMe (abbreviation of benzyloxycarbonyl lysine methyl ester) of the mycobactin core by Rv1347c (MbtK, GCN5 N- acetyltransferase). Finally, in the presence of NADPH and FAD, Lys-N6-hydroxylase [7], encoded by *mbtG*, performs the final N6-hydroxylation step at ϵ -amino position exclusively (VI).

Exochelin

The knowledge of the siderophore exochelin mostly originated from the study of the *M. smegmatis* exochelin. More than ten genes participate in exochelin biosynthesis. So far, no genes highly homologous to *fxbA*, *fxbB*, *fxbC*, *orf1*, *orf2*, *fxuA*, *fxuB* and *fxuC* can be found in *M. tuberculosis* genome [18], whether *M. tuberculosis* produces exochelin remains to be determined. The genes *fxbA*, *fxbB* and *fxbC* are required for exochelin biosynthesis [18, 39, 40]. FxbB and FxbC are homologous to non-ribosomal peptide synthetases and share their common motifs [40]. FxbB and FxbC possess six activation modules, FxbB1, FxbB2, FxbC1, FxbC2, FxbC3 and FxbC4, each of which contains an ATP-binding motif, an ATPase motif and a pantetheine attachment motif [18]. Three epimerase domains within C-terminal motifs

Fig. 1. Schematic representation of the synthesis of mycobactin in *Mycobacterium tuberculosis*. Abbreviations: "R" represents "H" or "OH"; "R'" represents "CnHn+1".

of FxbB1, FxbC1 and FxbC2 convert the *S*-amino acids to *R*-types [18]. Among other ORFs [18], ORF1, ORF2 and ORF3 share homology with the ABC transporters. ORF3 only contains a hydrophilic ATP-binding domain, while ORF1 and ORF2 have five and four hypothetical transmembrane domains in their N-terminal motifs apart from the ATP-binding cassettes. ORF4 and ORF5, with six and five transmembrane domains respectively, show homology to four putative membrane proteins of *M. tuberculosis* and *M. leprae* [18]. FxuD encoded by *fxuD* is a putative membrane-related receptor for ferric exochelin [18], while ExiT, a ABC transporter, is involved in the secretion of exochelin [40].

The exochelin is a linear formylated pentapeptide, which is organized with six unusual amino acids: one β -alanine, one (R)-allo-threonine, two (R)- δ -N-hydroxyornithines, one (S)- δ -N-hydroxyornithine and a formyl group. The R-amino acids in the siderophore are converted by three epimerase domains in FxbB and FxbC. The exochelin biosynthesis is proposed as following [18]: the FxbB1 and its C-terminal that contains an epimerase domain upload the first (R)- δ -N-hydroxyornithine, FxbB2 and FxbC3 (or FxbC4) may be responsible for attaching the β -alanine and the (S)- δ -N-hydroxyornithine to the backbone, the third and fourth R-amino acids, (R)- δ -N-hydroxyornithine and (R)-allo-threonine, are packed by the FxbC1 and FxbC2 and their C-terminal epimerase domains, the FxbA probably transfer a formyl group to the first (R)- δ -N-hydroxyornithine. The exochelin secreted by mycobacteria is a pentapeptide. The evolution of this molecule remains a mystery.

Comparative genomic analysis of mycobacterial siderophore biosynthetic enzymes

As Table 2 shows, fatty acyl synthetases responsible for side chain biosynthesis and similar functional enzymes exist in nearly all mycobacteria (except *M. abscessus*). Compared with the virulent *M. tuberculosis* H37Rv, the avirulent strain *M. tuberculosis* H37Ra has nearly all mycobactin/carboxymycobactin synthases with 99-100% identity, as is *M. bovis* AF2122/97. While the attenuated *M. bovis* BCG str. Pasteur 1173P2 lacks MbtI, MbtA and MbtG and the side-chain synthases also have low identity. This might underlie the attenuation of this BCG. *M. smegmatis* MC² 155, a fast-growing mycobacteria bearing all similar functional enzymes with identities between 30-80%.

The widespread of these biosynthetic enzymes in nearly all pathogenic mycobacteria implicates that mycobactin might be involved in pathogenesis. The role of multiple MbtH and FadD33 in *M. avium subsp. para* K-10 remains to be determined and is suggestive of functions in addition to siderophore biosynthesis.

Non-pathogenic mycobacteria, *Mycobacterium sp.* JLS, *Mycobacterium sp.* KMS, *Mycobacterium sp.* MCS, *M. vanbaalenii* PYR-1 and *M. gilvum* PYR-GCK, have low, even no, identities of mycobactin core synthetases while the side-chain biosynthesis enzymes are conserved with identity between 60% and 80%. However, they can utilize xenosiderophores by means of unknown mechanisms [41]. For example, *M. leprae* TN, incapable of producing mycobactin by itself, can usurp siderophores from other microbes, such as the *M. neoaurum* exochelin MN [25].

Regulatory mechanisms of mycobacterial siderophore biosynthesis

Mycobacterial siderophore biosynthesis is intensively regulated, largely by iron levels. Under low iron concentrations, siderophores are abundantly produced to obtain iron. The siderophore biosynthesis pathway is shut off in the presence of high iron. Four potential iron-dependent regulators have been found in *M. tuberculosis* [35]: FurA and FurB of the Fur family, IdeR and SirR of the DtxR family.

IdeR's role in mycobacterial iron metabolism is well documented and regulates about one third of iron-regulated genes [42]. IdeR, a pleiotropic regulator, plays a negative role in siderophore biosynthesis and modulates iron storage positively under high-iron conditions. It is also involved in the oxidative stress response, in both *M. tuberculosis* and *M. smegmatis* [42, 43]. Similar to DtxR, the presence of ferrous iron will enable the metal-IdeR complex to bind to the regulatory region of *fxbA*, *mbtA-mbtB* and *mbtI* to block their transcription [44, 45]. Other divalent metals (Mn, Zn, Co) allow IdeR to bind to these regulatory regions, in biochemical experiments [44, 45]. IdeR also binds to a 212bp sequence upstream of the Rv1348 transcription site [45], suggesting an effect on the siderophore transport. The iron metabolism-related genes regulated by IdeR are summarized in Table 3 [42].

In addition, Irep-28, the DNA-binding HU homologue HupB protein (hupB, Rv2986c) in the cell wall, has coordinated expression with siderophores mycobactin(s) and

Mycobacteria		Enzymes	Function	Mycobacterium tuberculosis H37Ra															Remarks
GenBank	Accession			Gene	Protein	Accession	Gene	Protein	Accession	Gene	Protein	Accession	Gene	Protein	Accession	Gene	Protein	Accession	
MdH	(R2386c)	Isoschironate synthase/salicylate synthase	100	100	100	79	74	65	no	74	NO	"32.a"	"32.a"	"32.a"	"31.a"	"32.a"	a=anthranilate synthase component I		
MdJ	(R2385)	Acetyl hydroxylase	34	100	100	75	"69.a"	"48.b"	"34.lipase lipol"	"68.lipase"	100	"38.a"	"38.a"	"38.a"	"29.a"	"40.b"	a=β-hydroxylase domain-containing protein; b=putative lipase/esterase		
MdA	(R2384)	Salicyl-AMP ligase/salicyl-S-ACP synthetase	100	100	100	73	"79.A"	64	No	258/433=78	NO	"67.B"	"67.B"	"67.B"	"64.B"	"66.B"	"a=2,3-dihydroxybenzoate-AMP ligase; b=AMP-dependent synthetase and ligase"		
MdR	(R2383a)	Peptide synthetase, editing the acyl carrier protein (ACP)	"100.99"	99	99	50	71	51	100	71	99	"61.A"	"61.B"	"61.B"	"59.A"	"57.A"	A=amino acid adenylation domain-containing protein; B=amino acid adenylation		
MdC	(R2382c)	Polyketide synthase	48	100	100	51	"72.a"	50	48	72	100	78	78	78	"79.b"	"72.b"	"a=erythronolide synthase, modules 1 and 2; b=ketosynthase synthase"		
MdD	(R2381c)	Polyketide synthase	36	100	100	38	45	38	"36.hydroxylated protein"	46	100	"55.1"	"55.1"	"55.1"	"52.1"	"48.1"	I=acyl transferase region		
MdE	(R2380a)	Peptide synthase	33	100	100	44	"66.b"	51	100	66	100	"71.a"	"71.a"	"71.a"	"71.a"	"68.a"	a=amino acid adenylation domain-containing protein; b=linear gramicidin synthetase subunit B; c=putative sulfolipase		
MdF	(R2379c)	Peptide synthase	100	99	99	42	"60, c"	42	99	61	99	"66.a"	"66.a"	"66.a"	"52.a"	"61.a"	a=HD dependent oxidoreductase		
MdG	(R2378a)	Acetyl hydroxylase	100	100	100	67	89	71	No	88	NO	"76.a"	"75.a"	"75.a"	"76.a"	"76.a"			
MdH	(R2377c)	Unknown	100	99	99	75	79	75	No	79	99	78	78	78	71	78			
MdL	(R2344)	Acyl carrier protein	68/68=100	100	100	23/56=42	55/67=83	"19/56=34, MAB_1691"	18/42=43	55/67=83	18/42=43	50/64=79	50/64=79	50/64=79	"43/54=80.a"	45/64=71	a=phosphopantetheine-binding protein		
MdM	(R2345)	Acyl-AMP ligase/acyl-CoA synthase	100/35	99	100	36	71	36	100	70	100	64	64	64	63	61			
MdN	(R2346)	Acyl-ACP dehydrogenase	100	100	100	82/295=29	83	27	26=76/296	82	29	79	79	79	80	"92/303=3.1.1"	I=acyl-CoA dehydrogenase domain-containing protein		
MdK	(R2347c)	GCN5 N-acetyltransferase	"28, MRA_1602"	100	100	59	"82.58"	43	99	"88MAP155 2.57MAP3 1.49c"	"2380C, 1630c"	66	66	66	"65.A"	63	A=malonyl CoA decarboxylase		
IrA	(R2348)	Drug-transport transmembrane ATP-binding protein ABC transporter	99	100	100	77	80	63	No	"80.MAP24 1.4c"	NO	"123/270=46a"	"122/271=46a"	"122/271=46a"	120/241=50	166/433=38	a=ABC transporter related		
IrB	(R2349)	Drug-transport transmembrane ATP-binding protein ABC transporter	110/241=46	100	100	79	77	57	No	78	NO	"118/317=38a"	"118/318=38a"	"118/318=38a"	"128/324=40a"	"127/318=40a"			
BfrA	(R2352)	Bacterioferritin [72]	"43, a"	100	100	"43, a"	"44, a"	"44, a"	No	"44, a"	"43, a"	"43, a"	"43, a"	"43, a"	"44, a"	"44, a"	a=succinate dehydrogenase flavoprotein subunit		
BfrB	(R2341)	Ferritin [72]	100	100	100	90	34/151=23	112/166=68	No	34/151=23	NO	120/164=74	120/164=74	120/164=74	111/156=72	107/156=69			

Table 2. Comparative genomic analysis of mycobacteria siderophore biosynthesis enzymes. BLAST IN NCBI (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi)

Table 3. IdeR regulating iron metabolism-related genes, gene products and the regulation [42]. ^a, according to reference [55]; ^b, conserved hypothetical protein.

Rv no.	gene	gene products	regulation
0288		CHP ^b , ESAT-6 family	inhibited by iron and IdeR
1345	<i>fadD33</i>	Acyl-CoA synthase	
1346	<i>fadE14</i>	Acyl-CoA dehydrogenase	
1347c	<i>mbtK</i>	IucB-like protein	
1348	<i>irtA</i> ^a	YbtP-like protein	
1349	<i>irtB</i> ^a	YbtQ-like protein	
2377c	<i>mbtH</i>	Mycobactin synthesis	
2378c	<i>mbtG</i>	Lysine-N-oxygenase mycobactin synthesis	
2379c	<i>mbtF</i>	Peptide synthase, mycobactin synthesis	
2380c	<i>mbtE</i>	Peptide synthase, mycobactin synthesis	
2381c	<i>mbtD</i>	Polyketide synthase, mycobactin synthesis	
2382c	<i>mbtC</i>	Polyketide synthase, mycobactin synthesis	
2383c	<i>mbtB</i>	Peptide synthase, mycobactin synthesis	
2384	<i>mbtA</i>	Salicylate-AMP ligase, mycobactin synthesis	
2385	<i>lipK</i>	Probable acetyl-hydrolase	Iron- and IdeR-induced
2386c	<i>mbtI</i> (<i>trpE2</i>)	Isochorismate synthase, mycobactin synthesis	
1552	<i>bfrA</i>	Bacterioferritin	
3841	<i>bfrB</i>	Ferritin-like protein	
0338c		Fe-S protein	Repressed by Iron but IdeR-independent
0009	<i>ppiA</i>	peptide-prolyl-cis-trans isomerase	
1463		Probable ABC transporter	
2794c		CH, similar to proteins involved in vibriobactin and enterobactin synthesis	
1908c	<i>KatG</i>	Catalase-peroxidase	Iron-induced but IdeR-independent
3145	<i>nuoA</i>	NADH dehydrogenase chain A	
3146	<i>nuoB</i>	NADH dehydrogenase chain B	
3147	<i>nuoC</i>	NADH dehydrogenase chain C	
3148	<i>nuoD</i>	NADH dehydrogenase chain D	
3152	<i>nuoH</i>	NADH dehydrogenase chain H	
3153	<i>nuoI</i>	NADH dehydrogenase chain I	
3155	<i>nuoK</i>	NADH dehydrogenase chain K	
3156	<i>nuoL</i>	NADH dehydrogenase chain L	
3157	<i>nuoM</i>	NADH dehydrogenase chain M	
3158	<i>nuoN</i>	NADH dehydrogenase chain N	
3246	<i>mtrA</i>	Two-component response regulator	

carboxymycobactin(s), in response to iron level [46]. Seven IRPs (iron regulated proteins) identified by Calder and Horwitz [47] are regulated by iron concentrations: Irp15, Irp24 and Irp29 are upregulated in rich iron milieu, while Irp10, Irp13, Irp23 and Irp28 expression increased under iron deficiency. *M. neoaurum* mycobactin exochelin MN was expressed concomitantly with a 21-kDa envelope protein, and their levels are regulated by iron [48]: when iron is limited, both two components are expressed. When the iron is abundant, their levels are lowered. But the threshold of iron might vary with strains, medium and growth stages.

Siderophore-mediated iron transport mechanism

Microbial iron uptake process is largely as following: soluble siderophores are secreted to obtain environmental or host iron and are transformed into ferric siderophores. The ferri-siderophores are then transported into cells by the receptors located in the cell envelopes, and then iron ions are delivered to bacterioferritin to satisfy the physiological requirements. Both exochelins and carboxymycobactins can seize the extracellular iron and transfer the irons to the intracellular siderophores. However, as shown in Fig. 2, irons transport across the cell membranes are different.

Fig. 2. Schematics of carboxymycobactin (A) and exochelin (B) transporting iron pattern [8]. (A) Extracellular desferrated-carboxymycobactins plunder the iron from host iron-binding proteins and convert to ferri-carboxymycobactins. Then the carboxymycobactin-iron complexes transferred iron to non-ferrated mycobactins associated with the bacterium cell wall to form ferrated mycobactins. Fe^{3+} ions are transferred to cell membrane by ferri-mycobactins, converted to Fe^{2+} by the reductases there and released into cytoplasm. At last, the excess irons could be stored or involved in physiological process.

(B) Ferri-exochelins traverse the cell envelope, then pass through the tetramers which composed of one FxuA, two FxuB and one FxuC, and release the ferric ions into the cytoplasm. Subsequently, the desferrin-exochelins are exported by ExiT proteins to chelate host iron. After being reduced in cytoplasm, the Fe^{2+} binds to various apoproteins and converts them to functional proteins, the excess iron ions would be saved in mycobactins transiently or stored in bacterioferrins. Abbreviations: CM, carboxymycobactin; E', exochelin; F, ferri; F-CM, ferri-carboxymycobactin; F-E', ferri-exochelin; M, mycobactin; ?, putative components involved in the transport.

The intracellular iron level is coordinated by salicylate and IdeR [49]. In iron-limited conditions, *M. tuberculosis* regulates the production of mycobactin and carboxymycobactin and carboxymycobactin secretion. As Fig. 2A shows, extracellular desferrated-carboxymycobactins can plunder the iron from the host iron-binding proteins, such as transferrins, lactoferrins and even the "iron storage" ferritins, and convert to ferri-carboxymycobactins. Then the carboxymycobactin-iron complexes transferred iron to non-ferrated mycobactins associated with the bacterium cell wall to form ferrated mycobactins [50]. Fe^{3+} ions are transferred to cell membrane by ferri-mycobactins, converted to Fe^{2+} by the reductases there and released into cytoplasm. With the help of cytoplasmic salicylate, iron is transferred into apoproteins and porphyrins to function or bacterioferritins to store [49], wherein over ninety percent of iron is stored [41]. IrtA facilitates the iron-unloaded mycobactins to back cell wall and recycle the iron trafficking [51]. In brief, mycobactins can cache excess iron, but the ultimate storages are bacterioferritins [41].

The iron transfer mediated by exochelins [40, 52] is illustrated in Fig. 2B: ferri-exochelins are recognized by the specialized receptors in the cell wall and can traverse

the cell envelope. Subsequently, the molecules pass the tetramers consisting of one FxuA, two FxuB and one FxuC, and release the ferric ions into the cytoplasm. Subsequently, the desferrated-exochelins are exported by ExiT proteins to chelate environmental iron. After being reduced in cytoplasm, the Fe^{2+} binds to various apoproteins and converts them to functional proteins, the excess iron ions would be saved in mycobactins transiently or stored in ferritins/bacterioferrins.

Although Gram-negative bacteria have various mechanisms for siderophore secretion and uptake across the outer membrane, analogous systems for penetrating the substantial cell wall of mycobacteria are poorly defined [53]. IrtA and IrtB, two putative ABC transporters located in cell membrane, are involved in the uptake of carboxymycobactin and that IrtA, which has a Flavin-Adenine Dinucleotide-binding domain and has putative Fe reductase activity, indicating IrtA functions to couple iron transport and assimilation [54, 55]. These two molecules interact and synergize to maintain the balance between iron and siderophores within mycobacterial cells [51]. And inactivation of the IrtAB system decreases the ability of *M. tuberculosis* to survive in mouse lung and macrophages [55]. Homology searches indicated that *M. smegmatis* genes namely *msmeg_6554* and *msmeg_6553* might be the counterparts of *M. tuberculosis* *irtA* and *irtB*, respectively [51]. Knockout of *msmeg_6554* resulted in an impaired *M. tuberculosis* siderophore export that is restored upon complementation with *M. tuberculosis* *irtA* [51]. So *Msmeg_6554* may be involved in siderophore secretion.

ESX-3 is a specialized secretion system required for mycobactin-mediated iron acquisition [56]. The ESX-3-deficient mycobacteria can synthesize mycobactin but incapable of utilizing the bound iron and are impaired severely for growth during macrophage infection [56]. Moreover, ESX-3 is regulated by the availability of iron and zinc, as part of the *ideR* and *Fur* (also known as *Zur*) regulons [42, 57].

In addition, the porins in the cell envelope of *M. tuberculosis* and two-component responders Irep-Mta72 probably participate in the trafficking of iron [58, 59]. Porins played a role in the soluble iron acquisition under iron abundance and might affect the *M. smegmatis* siderophore production under iron deficiency [60]. Furthermore, investigators found that mycobacteria have taken advantage of endogenous macrophage mechanisms for iron mobilization and lipid sorting for iron acquisition during infection [61]. After bacteria infection, lipid droplets within human macrophage would concentrate into phagosomes and change the phagosomal contents [62, 63]. Ironically, mycobacteria can benefit from this host defense mechanism to deliver iron during infection. This discovery suggests that mycobacteria can hijack macrophage inherent strategies to maintain their iron homeostasis [61].

Future perspectives

Significant advances on siderophore biosynthetic pathways and siderophore-mediated iron transport mechanism in different species of mycobacteria have revealed that iron acquisition, assimilation and regulation in *M. tuberculosis* play crucial role in proliferation, virulence and latency. Thus the components involved in this process are attractive target candidates for the development of inhibitors as potential new antibiotics for treating tuberculosis.

The relevance of mycobactin synthesis for pathogenic mycobacterial growth [64, 65] highlights the enzymes involved in siderophore production as potential target candidates for exploring the development of novel anti-tuberculosis. PAS or p-aminosalicylic acid, one of the earliest drugs to be used for tuberculosis treatment, is an analog of salicylic acid and inhibits the conversion of salicylate to mycobactin [49]. In an attempt to block siderophore synthesis, Qiao et al. [66] investigated the structure-activity relationship of 5'-O-[N-(salicyl) sulfamoyl]adenosine a potent inhibitor of the mycobactin biosynthetic enzyme MbtA (encoded by the gene *Rv2384*). The hydroxy group is essential for an optimal inhibitory activity via comparison of diverse derivatives. On the other hand, Somu et al. [67] have

designed, synthesized, and evaluated a series of bisubstrate inhibitors of the adenylate-forming enzyme MbtA involved in biosynthesis of mycobactins. An acylsulfamide was found to be potent inhibitor of *M. tuberculosis* growth under iron-limiting conditions.

IdeR is an essential regulator with a major role in controlling iron metabolism. The essential nature of IdeR makes it a potential candidate for therapeutic intervention against tuberculosis [42, 68]. Moreover, investigating the role of IdeR-regulated genes in iron acquisition should provide additional potential targets for chemotherapy [69, 70]. Targeting the *M. tuberculosis* specific iron transport and acquisition pathway involving ESX-3 or IrtA/IrtB-Rv2895c could be another promising strategy to selectively block *M. tuberculosis* iron assimilation without impairing the host iron uptake machinery [51, 56].

Although great strides have been achieved as to siderophores, many remain to be explored. For instance, whether more secretion systems beyond ESX and IrtAB are involved in the siderophore utilization, siderophore-mediated iron acquisition needs some extracellular components to assist and what their function mechanisms are, for gram-positive bacteria, *esp. M. tuberculosis*, their impervious heavy waxy coats is a formidable barrier for novel antibiotics. Siderophore might be harnessed to chaperone or facilitate the administration of the antibiotics.

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