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Glycogenomics of *Mycobacterium tuberculosis*

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Abstract

Glycogen is an important energy store of almost all living organisms. It is an alpha linked polymer comprised of thousands of glucose units. In bacteria it is usually synthesized when carbon ions are in excess in the growth medium and its synthesis helps for the survival of the bacteria under such nutritional conditions. Mycobacterium tuberculosis (*M. tuberculosis*), accumulates glycogen during the adverse condition such as reactive oxygen and nitrogen intermediates, low pH, nutrients and other vital element starvation for their survival in the host. Glycogen also plays a very important role in the pathogenesis of *M. tuberculosis*. The biosynthesis of glycogens is mediated by glycosyltransferases enzyme which can be divided into two families; glycogen transferase (GT) 3 and glycosyltransferases GT 5. Regulation of glycogen metabolism in bacteria involves a complex mechanism, involving several synthase enzymes such as glycogen synthase A (glgA), glycogen branching enzyme (glgB), and catalytic enzyme (glgC). Another enzyme known as glycogen phosphorylase (glgP), removes extra units of glucose from the non- reducing ends of the glycogen molecule. Several workers have recognized role of glycogen in Mycobacterial pathogenesis, in the recent years. Trehalose-dimycolate (TDM) and trehalose-monomycolate (TMM) present in the cell wall are indeed a precursor of mycolic acid of Mycobacteria, which plays an important role in its invasion and pathogenesis. This review focuses on various cycles and mechanisms involved in the glycogen synthesis in *M. tuberculosis* and its role in pathogenesis.

Keywords: Glycogen; *Mycobacterium tuberculosis*; MTB; Glycogen transferases

Introduction

All living organisms present on the earth accumulates glucose as energy storage molecules in the form of glycogen or starch [1,2]. Glycogen is a polysaccharide localized in the cytoplasm, which is mainly utilized by bacteria, fungus or animals [3-5]. Starch is synthesized in the plant and stored in plastids. It is composed of two glucose polymers: amyl pectin, which is the main component and is sufficient to form starch granules, and amylase [6]. Glycogen and amylopectin are the complex molecules containing α-4-linked glucose units with α-6-branching points. The length and number of branches vary depending on the organism [5,7,8]. Glycogen is linked with α glucose polymer with the ~90 % α-4-links in its backbone and ~10 % α-6-linked branches [9]. It is comprised thousands of glucose units and is generally synthesized in bacteria, when excess carbon is present over other nutrient that limits growth [3,10]. Glycogen covered 60% of dry cell weight and enhances the cell survival (e.g. *M.tuberculosis*). It rapidly accumulates prior to beginning of sporulation in Bacillus cereus and production of exo-polysaccharides in *M. tuberculosis* [11].

Glycogen and starch both are extra-large sized glucose polymers and are the major reservoir of freely available carbon and energy source of all living organisms such as archaea, eubacteria, yeasts and higher eukaryotes including animals and plants. The parasitic lifestyle appears to be related to the reduction and eventual complete abolishment of glycogen metabolism [12]. In mammals, uptake and utilization of glucose are under stretched control. Any defect in the normal glucose level, lead to induce a variety of glycogen storage diseases, like diabetes in the human [13].

M. tuberculosis, aerobic, acid fast bacilli that cause tuberculosis in human, accumulates glycogen during the adverse condition i.e. reactive oxygen and nitrogen intermediates, low pH, nutrients and other vital element starvation for their survival in the host [14]. Although, glycogen accumulation does not occur during exponential growth under laboratory culture conditions, but existence of glycogen may increase the viability of *M. tuberculosis* under adverse conditions. Glycogen also plays a very important role in the pathogenesis of *M. tuberculosis* [15]. It has been validated in the *M. tuberculosis*, if the organisms are physiologically inactive for long time period; its storage of sugars becomes very important for survival. Various groups of scientific community has been reported that, glycan's may regulate biochemical pathways by binding of these molecules to proteins and lipids during the post-translational modifications via covalent and non-covalent interactions. It also acts as a boundary between cells, tissues and organs to organize biological processes [16]. Therefore, from a biological viewpoint, complex glycan's represent a promising, but relatively untapped, source for the development of new pharmaceutical agents. In this context, many uncharacterized glycosyltransferase (GTs) of *M. tuberculosis* are of particular interest of researchers. This review summarizes present knowledge and facts on characterizing and putative GTs in Mycobacterium spp.

Glycosyltransferases (GTs)- A key enzymes of glycogen synthesis

The biosynthesis of glycogen is mediated by glycosyltransferase enzyme [17]. GTs constitutes a large family of enzymes that are involved in the biosynthesis of oligosaccharides, polysaccharides and glycoconjugates [18,19]. Due to enormous diversity of these enzymes, it's mediating a wide range of functions both structural and storage for molecular signaling. It is present in both prokaryotes and eukaryotes

and generally display vast specificity for both the glycosyl donor and acceptor. In eukaryotes, glycosylation reactions occur in the specialized compartment such as golgi apparatus and generate a wide range of structural oligosaccharide diversity of eukaryotic cells [20].

But in prokaryotes, they produce a variety of glycoconjugates and polysaccharides of vast structural diversity and complexity. In E. coli, glgC, glgA and glgB gens encode glycogen synthesis enzymes and glgP and glgX genes encode enzymes for glycogen degradation. The role of glgC and glgA is a generation of the activated glucose nucleotide diphosphate and linear glucan chain respectively. GlgB, or glycogen branching enzyme, catalyzes the transfer of a fragment of 6–7 glucose units from a non-reducing end of hydroxyl group of C6 of a glucose unit, either on the same glucose chain or adjacent chains. GlgB is very essential enzyme present in bacteria [21], and fungus, responsible for glycogen accumulation. Functional glgB (encoded by the ORF Rv1326c) is essential for normal growth of *M. tuberculosis* [22]. In bacteria, glycans includes many unusual sugars which are generally not found in vertebrates, i.e. Kdo (3-deoxy-D-manno-octulosonic acid), heptoses, and also various modified hexoses. The modified hexoses molecules play a very important role in the pathogenicity of bacterial cells. In some instances, the donor substrates are lipids (dolichol-phosphate), linked to glucose or mannose or a dolichol-oligosaccharide precursor and play major role in the assembly of peptidoglycan, lipopolysaccharide, and capsules [17].

Classification of glycosyltransferase (Glycogen Synthase)

Based on sequence and structural analysis, glycogen synthase (GS) have grouped within the GTB-fold of glycosyl transferases. These structures are characterized by the presence of two Rossmann fold domains with a deep inter domain cleft in between that harbors the substrate-binding and catalytic sites. GTB-fold enzymes are further subdivided into two families, GT3 and GT5 (Figure 1). The bacterial and archaeal GS enzymes are grouped in the GT5 family and eukaryotic enzymes are grouped into the GT3 family and are regulated through the allosteric activator glucose-6- phosphate (G-6-P) and inhibitory phosphorylation.

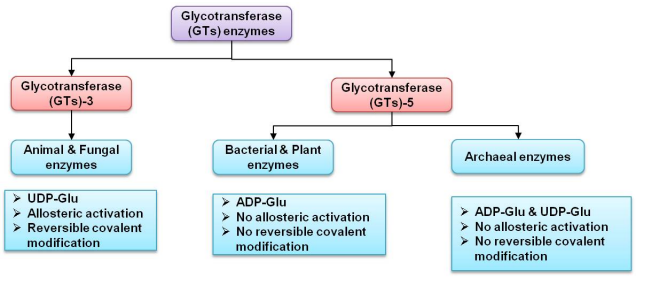

Figure 1: Schematic representation of the Glycotransferases (GTs) enzymes classification.

An additional point of distinction is that the bacterial enzyme uses adenosine diphosphate (ADP) -glucose as their sugar donor, whereas eukaryotic enzymes almost exclusively utilize uridine diphosphate (UDP) glucose as their donor molecule. Archaeal enzymes are capable of using both ADP and UDP-glucose as sugar donors. To date, three dimensional structures have been determined for three members of the GT5 family - a monomeric *E. coli* enzyme, dimeric Agrobacterium tumefacians and trimeric Pyrococcus abyssi. However, these structures have shed little light on the regulatory mechanisms controlling eukaryotic enzymes.

Mechanisms of action

The action mechanisms of GTs are based on the use of an activated donor, such as nucleotide di-phosphosugar, nucleoside mono-phosphosugar or lipid phosphosugar and acceptor molecules like a hydroxyl group of amino acid. GTs catalyzes the transfer of monosaccharide moieties from activated nucleotide sugar (glycosyl donor) to glycosyl acceptor molecules, forming glycosidic bonds. The mechanism of inverting GTs is believed to be similar to the one of inverting glycosyl-hydrolases with the requirement of one acidic amino acid, which activates the acceptor hydroxyl group by deprotonation [20].

c glycogen- role and regulation

In higher eukaryotes such as mammals, glycogen is synthesized at the time of nutritional abundance. The two major tissues or organ systems that serve as a glycogen stores in higher eukaryotes, are skeletal muscles and liver. Other organs like brain, adipose, kidney and pancreas are also capable of synthesizing minute quantity of glycogen. In the skeletal muscles, glycogen provides energy for muscular contraction during generation of glucose-6-phosphate (G-6-P) from glycogen for entry into glycolysis as a means for ATP production and liver glycogen play vital role in glucose homeostasis or maintaining the blood glucose level during fasting. Any defect or mutation in the enzymes involved in glycogen metabolism leads to development of glycogen storage disease (GSD), which affects the liver, muscle or both and other organs.

In the budding yeast (Saccharomyces cerevisiae), glycogen is one of the major reservoir of carbohydrate and accounts 20% of the dry weight of yeast cells. The amount of glycogen accumulated in the cell increases, when the cell enters into the stationary phase or in depletion of essential nutrients like nitrogen and phosphorous in the growth media. Also, Glycogen accumulation was observed when exponentially growing *S. cerevisiae* exposed cells to high temperature, salt, oxidizing agents or ethanol. The accumulated glycogen has been utilized for their survival by yeast during nutrient deprivation [23,24]. Additionally, yeast has a growth advantage over other non-glycogen accumulative cells, as it makes a contribution to the overall fitness of the cell [25].

The glycogen regulation in eukaryotes is controlled by the activity of various enzymes such as glycogenin, glycogen synthase, branching and de-branching enzyme followed by multiple mechanisms including covalent modification, allosteric activators and translocation within the cells [23,25-29]. The common regulatory themes of GTs are phosphorylation and allosteric activation by G-6-P, but the physiological responses that interrupt these regulatory controls often differ between different organisms and even between different tissues of the same organism [29]. Yeast has two different isoforms of glycogen synthase (GS), of which the nutritionally regulated isoform-2 (GSY2) has shown to be the most essential enzyme for the accumulation of glycogen in the cells. Unlike the other higher eukaryote, where the regulation of glycogen metabolism is primarily control by the action of enzyme activities, in yeast it involves both transcriptional and enzymatic responses. The transcriptional response is dependent on the presence of the cis-element stress response element (STRE) in the promoter of the genes involved in glycogen

pathways. The enzymatic control of glycogen deposition is controlled by the activation of GS through G-6-P and inactivation of Glycoside-Pentoside-Hexuroni (GPH) through phosphorylation. Exposure of the starved cells to nutrients activates GPH, inhibits GS resulting in the mobilization of glycogen and vice versa.

Organisms	Organism Name	Enzyme Name	Name of Glycotransferese	GT Family	References
Virus	T4- Phage	BGT	β-Glycosyltransferase	GT63	[69]
Prokaryotes	A. tumefaciens	AtGS	Glycogen Synthase	GT5	[15]
	Amycolatopsis orientalis	GtfA	B-Epi-vancosaminyl transferase	GT-1	[70]
		GtfB	β-Glycosyltransferase	GT-1	[70]
		GtfD	β -vancosaminyl transferase	GT-1	[70]
	B. subtilis	SpsA	Putative glycosyltransferase	GT-2	[71]
	Campylobactor jejuni	CstII	α-2-3-8-Sialyltransferase	Gt-42	[72]
	E. coli.	MurG	β -1-4- Galactosyltranserase	GT-28	[73]
		OtsA	Trehalose-6-phosphate synthase	GT-20	[74]
		RfaF	Heptosyl transferase	GT-9	[74]
	Neisseria meningitidis	LgtC	α -1-4-Galactosyltransferase	GT-8	[75]
	Rhodothermus marinus	MGS	Mannosylglycerate	GT-78	[76]
	Mouse	Ext12	α-1-4-N- Acetylhexosaminlytransferase	GT-64	[18]
		ppGalNAc-T1	Polypeptide- α-GalNAc transferase	GT-27	[18]
	Rabbit	Glycogenin	α-Glucosyltransferase	GT-8	[74]
		GnT1	β-1-2-GlcNAc transferase	GT-13	[77]
	Bovine	α3GalT	α-3-Galactosyltransferase	GT-6	[78]
		β4GalT1	β-1-4- Galactosyltransferase I	GT-7	[78]
	Human	GlcAT-I	β-1-3-Glucuronytransferase	GT-43	[19]
		GlcAT-P	β-1-3-Glucuronytransferase	GT-43	[79]
		GTA	α-3-GalNAc transferase A	GT-6	[80]
		GTB	α-3-GalNAc transferase B	GT-6	[80,81]

Table 1: Glycotransferases enzymes with available crystal structures.

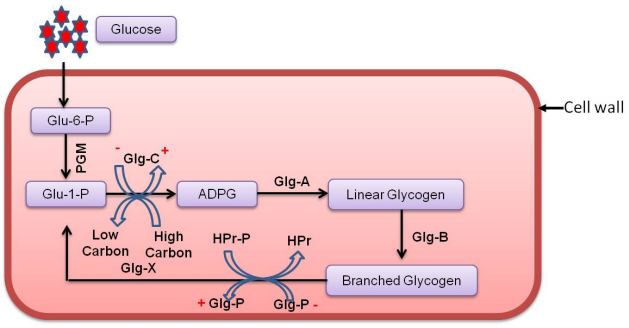

Figure 2: Systemic representation of genes mediated regulatory pathway of Glycogen synthesis in *M. tuberculosis*.

Prokaryotes glycogen- synthesis, degradation and regulation

The enzymology of glycogen biosynthetic and degradative processes is highly conserved in prokaryotes [3,30]. Extracellular glucose is taken up and converted into G-6-P by the carbohydrate phosphotransferase system (PTS). G-6-P is further converted into glucose-1-phosphate (G-1-P) by the action of phosphoglucomutase (PGM) and finally converted into ADP glucose (ADPG) in the presence of Mg^{2+} and ATP [30]. ADPG act as sugar donor nucleotide for the production of bacterial glycogen by the action of glycogen synthase (glgA). After chain elongation by glgA, glycogen branching enzyme (glgB) catalyzes the formation of branched oligosaccharide chains having α-6-glucosidic linkages [3]. Genetic evidence of glycogen synthesis suggested that glgC is the sole enzyme catalyzing the production of ADPG [30,31].

Regulation of glycogen metabolism in bacteria, involves a complex group of factors that adjusted to the physiological and energetic status

of the cell [32,33], expression of corresponding genes and cell-to-cell communication [34]. At genomic level, several factors have been described to control the bacterial glycogen accumulation. In *M. tuberculosis*, it is subjected to allosteric regulation of enzymes [3,35]. The product of glgC gene is representing the signals of high carbon and energy contents within the cell, whereas the presence of inhibitors provides the signal at the low metabolic energy levels (Figure 2) [30]. Allosteric regulation of glgC has been extensively reviewed in recent years including structural and functional relationships of glgC, glgA and glgB [3,30].

Glycogen phosphorylase (glgP), which removes glucose units from the non-reducing ends of the glycogen molecule, shows strong and highly specific interaction between glgP and HPr (a PTS component) by surface plasmon resonance ligand fishing [32,35]. The binding of glgP to HPr is maximal, when HPr is totally phosphorylated and reduce activity of glgP during log phase of *M. tuberculosis* and vice-versa. It's assumed that activity of glgP is regulated by the phosphorylation status of Hpr and therefore allowing the accumulation of glycogen at the beginning of the stationary phase under glucose excess conditions (Table 1) [35,36].

Pathways of glycogen synthesis, degradation and its regulation in *M. tuberculosis*

Glycogen synthesis, an endergonic process and is synthesized from monomers of UDP-glucose. The genetic basis of glycogen synthesis and degradation has been extensively characterized in *E. coli*. In E. coli, glgC, glgA and glgB genes encode glycogen synthesis enzymes and glgP and glgX encode enzymes for glycogen degradation [37,38]. It is expected that bacteria have been synthesize glycogen using classical glgC-glgA pathway (Figure 3).

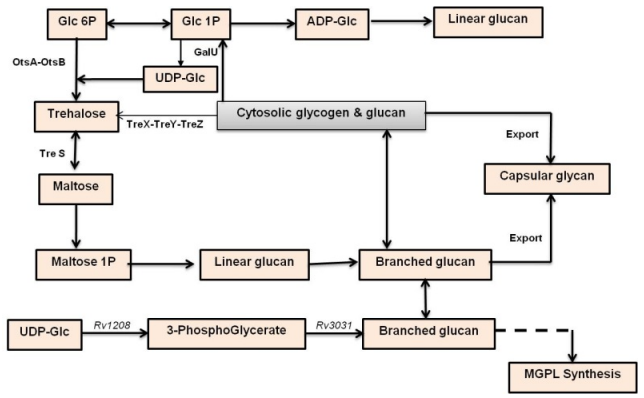

Figure 3: Bacterial glucan pathways. GlgC and GlgA are central to the classical glycogen pathway. The Rv3032 pathway is associated with methylglucose lipopolysaccharide biosynthesis. The newly identified GlgE pathway (red) (Kalscheue et al.) may contribute to cytosolic glycogen, capsular glucan and/or methylglucose lipopolysaccharide biosynthesis.

The activated glucose nucleotide diphosphate generated from G-1-P by the action of nucleotide di-phosphoglucose pyrophosphorylase (glgC) and subsequently polymerization by glycogen synthase (glgA), for generation of linear glucan [6,10]. Conversion of linear glucan's into glycogen is mediated by glgB enzyme through the transfer of oligo glucans (non-reducing-end) to the 6-position of residual chain for the generation of side branches [10,39].

Expressions of glgC and glgA genes are regulated by intracellular bacterial signals, which denote the energy status of the cell [40]. Deletion or mutations in glgC gene prevent glycogen synthesis in *E. coli*. [9]. The outcome of recent studies suggested that a tiny amount of glycogen can be synthesized in naturally glgC deleted mutant during growth under specific conditions [3,41]. Also, glgS is linked to the glycogen synthesis process, but role is still unclear. Recent study shows that it could be plays important role during glycogen accumulation in *E. coli* [4,38,42]. In prokaryotes, glycogen has been degraded by the combined action of two enzyme glgP (highly conserved enzyme together) and glgX, to yield G-1-P, which is directly utilized in the primary metabolism of bacteria [10]. Glycogen phosphorylase enzyme degrades glycogen by sequentially removes glucose units from the non-reducing ends of glycogen and glgX removes α- 6 linkages of glycogen via hydrolyzing manner [39]. glgP and glgX regulates glycogen degradation according to the energy requirement of bacteria. A recent study suggests the deletion of either glgP or glgX or both prevents degradation of internal stores of glycogen [39]. Trehalose, a well-known disaccharide present in bacteria as storage carbohydrate and is used as both an energy store and a stress-protectant. Trehalose helps bacteria to survive under desiccation, cold and osmotic stress [43,44]. Trehalose is consist of α-1-1 linkage of di-glucose and synthesized in bacteria from glucose phosphate intermediates via trehalose-6-phosphate, using the GalU-OtsA-OtsB system [45]. Trehalose can constitute more than 10% of cellular dry weight, and might be the major storage carbohydrate during specialized developmental states i.e. spores and bacteroids.

In mycobacteria, trehalose shows extraordinary interest for researchers due of its incorporation into mycolic acids. Mycolic acid is a cell wall component of mycobacteria and is involved in the pathogenesis of *M. tuberculosis* [46,47]. Because of poor appearance of trehalose, conversion from trehalose to glucose has been studies relatively low as compared to other molecules [48,49]. The transcriptional regulation of glycogen operon is also mediated through the RNA polymerase (Es70) by the restricted action of RpoS subunit [40]. Makinoshima et al. demonstrated that the rpoS mutants of E. coli accumulate less glycogen as compared to the wild type strain of *E. coli* [50]. The biosynthesis of glycogen is depending on the substrate accessibility and allosteric activity of ADP-glucose pyrophosphorylase [9] and catabolism is adjusted to accommodate changes in the availability of easily utilizable energy sources [40].

Role of glycogen under stress condition in *M.tuberculosis*

Mycobacterium cell wall accounts approximately 2-3% of dry weight of bacteria and constituted mostly of polysaccharide and proteins (94-99%). *Mycobacterial glycans* is similar to *E. coli* glycogen and the exact role of glycogen under stress (hypoxia, nutrient deprivation, Nitrous oxide treatment and growth in acidic media) environment is not fully understood [22]. But it has been reported by various group of scientific community, mycobacteria accumulates glycogen under stress condition for their survival and endogenous reserves during post exponential growth. [Antoine and Tepper, 1968 was demonstrated that glycogen and lipid accumulation increased affectedly as the nitrogen/ sulfur content of the medium was dropped in *M. phlei* and *M. tuberculosis* under stress conditions. In the absence of exogenous carbon substrate, these reserve substrates were utilized as carbon and energy source and continued growth of organism.

Glycogen inhibits phagocytosis of *M. tuberculosis* in macrophage and also takes part in host-pathogen interaction during pathogen entry in to the host [51].

Alternatively, glycogen or its intermediates also act as key role for production of two unusual cell wall constitutes i.e. 6-O-methylglucosyl-containinglipopolysaccharides (MGLP) and the 3-O-methylmannose polysaccharides, which plays regulatory role in fatty acid biosynthesis in *M. tuberculosis* [52].

Role of glycogen in pathogenomics of *M.tuberculosis*

Glycogen is one of the most important storage sugars in the living world. It is provides nutrition to the organism and plays a very important role during host pathogen interaction [15]. Under the nutrient limiting conditions, glycogen accumulation occurs in *M. tuberculosis* and their role in survival and pathogenesis is poorly understood.

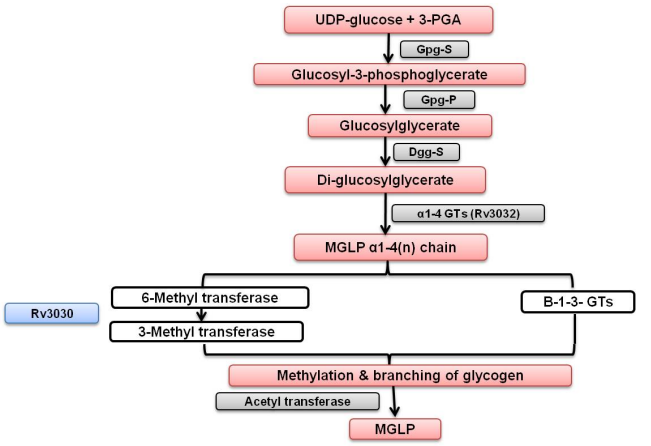

Figure 4: Pathway for the synthesis of the MGLP in *M. tuberculosis*. Confirmed activities are shaded in green. White boxes indicate putative/deduced enzyme activities. Genes linked to the MGLP pathway by mutagenesis studies are indicated in blue box. GpgS (Rv1208), glucosyl-3-phosphoglycerate synthase; GpgP (Rv2419), glucosyl-3-phosphoglycerate phosphatase; DggS, di-glucosylglycerate synthase; GT, glucosyltransferases

The glycogen has been playing a minor role in virulence and colonization in the *Salmonella typhi*, but has a more significant role in their survival. It has been demonstrated that the capsule consists of carbohydrate (glycan up to 80%), proteins and tiny volume of lipids [15,40,53]. The glycan's of mycobacterium envelope showed unique features than other bacteria. Its cell wall consists of mycolic acids (also known as arabinoglacton) and peptidoglycan, which constitutes "the core" of the cell wall and it is intercalated by a number of glycolipids such as lipoarabinomannan (LAM), the phosphatidylinositol containing mannosides (PIMs), phenolic glycolipids (PGLs), trehalose-dimycolate (TDM) and trehalose-monomycolate (TMM) present in the cell wall [27,54]. *M. tuberculosis* capsule is located outside of the mycolic acid layers, which contains generally polysaccharides such as arabinomannan and α-glucans and take part during the time of infection and invasion of macrophages [55]. The trehalose (formed by glycogen) is the precursor of formation of mycolyl acetyl trehalose (known as mycolic acid or cord factor). Also, mycobacteria synthesize unusual polysaccharides containing α-4-linked methylated hexoses (methyl glucose lipopolysaccharide (MGLP), methyl-mannose polysaccharide (MMP) that is slightly hydrophobic and helical conformation as amylose chain. These polysaccharides forms stable complex with fatty acids and modulate the activity of fatty acid synthase I (FAS I) In vitro. The MGLP has been found in both slow- and rapid-growing mycobacteria, while MMP has been detected only in rapid-growing mycobacteria. The synthesis and regulation of MGLP are shown in Figure 4. Based on presence of complex glucan and their derivatives in the *M. tuberculosis* cell wall suggested that glycogen might be responsible for pathogenesis.

Glyco-immunology in *M. tuberculosis* pathogenesis

Carbohydrate constitute *M. tuberculosis* capsules representing up to 80% of the extracellular polysaccharides (glycan), composed of α-4-α-DGlc-1 core branched at position six every five or six residues by 4-α-D-Glc-1 oligoglucosides [22,56,57]. The mycobacterial ligands that interact with macrophage receptors are less well characterized. Therefore, as the discovery of the role of capsular carbohydrates in bacterial pathogenesis, researchers have been given focus on the identification and characterization of the macrophage receptors involve in the binding and phagocytosis of *M. tuberculosis*. Carbohydrates are pathogenic mycobacterial species and have been determined much later than the discovery of the mycobacterial capsule [22,57,58].The reducing end of arabinogalacton (AG) consists of α-3-GlcNAc disaccharide, which is attached through phosphodiester linkage to the muramic acids of peptidoglycan [59]. The arabinan of AG contains 2 to 3 branched chain attached at 5-position to Galf residue of the galactan chain nearby to its reducing end. D-arabinan chain consists of 22 Araf residues [60]. The core structure of D-arabinan consists of backbone of α-5-linked Araf with several α-3-linked branch points and the non-reducing ends are always terminated by β-2-Araf. This assembly leads to the characteristic hexa-arabinoside (Ara6) motifs at the non-reducing ends of AG, of which the dimers [β-D-Araf-2-α-D-Araf] constitute mycolic acid attachment sites. PG and AG together forms an important covalently linked network located between the plasma membrane and the mycolic acid layer. These components of mycobacterial cell wall make the cell extremely robust and difficult to penetrate [55].

Unlike AG, LAM is a non-covalently linked to the cell envelope components and may be attached in the plasma membrane or mycolic acid layer or both through the phosphatidyl-myo-inositol (PI) unit. The reducing end of LAM shares structural similarities to the PI-mannosides (PIMs) and the inositol residues of the PI of both the PIMs and LAM are mannosylated at the 2 and the 6 positions (Figure 2) [55]. At present, there is limited information about the biological functions of these components. The mycobacterial cell wall moieties, i.e. lipoarabinomannan, binds to macrophage and glucans are able to inhibit the binding of mycobacteria to complement receptor 3 expressed in CHO cells [61]. The capsular polysaccharides, mediated the non-opsonic binding of *M. tuberculosis* H37Rv to CR3 [22,62]. The cell wall of *Pseudallescheria boydii* contains a vast amount of glycogen, which shows structural similarity to the *M. tuberculosis* and are involves in the infection or internalization of fungus by macrophages. It is also capable to induce the innate immune response by the involvement of toll-like receptor2, CD14 and MyD88 receptors [63]. In another study, the *M. tuberculosis* capsular components were revealed to contain compounds that displayed antiphagocytic properties with certain types of macrophages [61]. Also, induce

monocytes to differentiate into altered dendritic cells that failed to present lipid antigens to CD1-restricted T cells [64].

Glycogen based therapeutics and drug targets

The emergence of multidrug-resistant strains of *M. tuberculosis* accentuate the need to identify novel drug targets or new drugs for treatment of tuberculosis, which could act against the tubercular bacilli that persists during prolonged therapy with currently available drugs [65,66]. Enzymes involved in glycogen metabolisms (take part in synthesis of essential components of the cell envelope in bacteria), display auspicious drug targets for designing new drugs against mycobacteria; glgB shows unique drug targets for *M. tuberculosis*. It has been demonstrated that toxic polymers accumulated insight the glgB autotrophs and finally induce cell death. The absence of glucan's did not affect the outcome of macrophage infections with mycobacteria mutants, but its presence advise their protective role in persisting stage of mycobacteria during chronic infections [67]. Additionally, an alternative pathway (glgE depended) of glucan's biosynthesis was identified in mycobacteria. The glgE gene transfers an activated glucose residue to maltose1-phosphate via alpha 1-4 linkage. The gene pep2 (Rv0127) would phosphorylate and activate maltose reducing glucose and ultimately polymerization of glycogen initiated. As similar to glgB mutant role, mutation in glgE gene displays auspicious drug targets for mycobacteria, as it is part of earlier unrecognized α-glucan pathway that has never been targeted to induce death in mycobacteria. GlgE displays killing of bacteria by two mechanisms, The first death mechanism (glgE dependent) is self-poisoning by accumulation of the phosphosugar Maltose1phosphate followed by feedback inhibition of glgE. The second death mechanism (glgE independent) is based on essentiality of glgE pathway products. Both the genes (glgB and glgE) seem to be in an operon and it was assumed that the reason for their essentiality in mycobacteria was the accumulation of toxic product. Thus, inhibiting GlgE has become an exciting drug target [67].

Alternatively, Trehalose synthesis pathway from glycogen is widely studies in mycobacteria and the enzyme involved in trehalose metabolism shows promising drug targets for *M. tuberculosis* due to its importance in bacterial cytoplasm and presence in toxic glycolipids [66]. Several antibiotics, which inhibit the growth of *M. smegmatis* had an effect on the trehalose biosynthetic enzymes. Disruption of trehalose mycolyltransferase enzyme by 6-azido-6-deoxy-a,a-trehalose shows inhibition of mycobacterial growth in vitro [68].

In summary, glycotransferase enzymes, which are involved in the synthesis of essential components of the cell envelope in bacteria, could be explored as novel drug targets for the development of new drugs against bacterial pathogens.

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