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# Disruption of the SucT acyltransferase in *Mycobacterium smegmatis* abrogates succinylation of cell envelope polysaccharides

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Zuzana Palčková<sup>‡</sup>, Shiva K. Angala<sup>‡</sup>, Juan Manuel Belardinelli<sup>‡</sup>, Haig A. Eskandarian<sup>§</sup>, Maju Joe<sup>¶</sup>, Richard Brunton<sup>¶</sup>, Christopher Rithner<sup>||</sup>, Victoria Jones<sup>‡</sup>, Jérôme Nigou<sup>\*\*</sup>, Todd L. Lowary<sup>¶</sup>, Martine Gilleron<sup>\*\*</sup>, Michael McNeil<sup>‡</sup>, and Mary Jackson<sup>‡1</sup>

From the <sup>‡</sup>*Mycobacteria Research Laboratories, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado 80523-1682*, the <sup>§</sup>*Global Health Institute, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne VD, Switzerland*, the <sup>¶</sup>*Alberta Glycomics Centre and Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada*, the <sup>||</sup>*Central Instrumentation Facility, Department of Chemistry, Colorado State University, Fort Collins, Colorado 80523-1872*, and the <sup>\*\*</sup>*Institut de Pharmacologie et de Biologie Structurale, Université de Toulouse, CNRS, UPS, 205 Route de Narbonne, 31077 Toulouse, France*

Edited by Gerald W. Hart

Similar to other prokaryotes, mycobacteria decorate their major cell envelope glycans with minor covalent substituents whose biological significance remains largely unknown. We report on the discovery of a mycobacterial enzyme, named here SucT, that adds succinyl groups to the arabinan domains of both arabinogalactan (AG) and lipoarabinomannan (LAM). Disruption of the SucT-encoding gene in *Mycobacterium smegmatis* abolished AG and LAM succinylation and altered the hydrophobicity and rigidity of the cell envelope of the bacilli without significantly altering AG and LAM biosynthesis. The changes in the cell surface properties of the mutant were consistent with earlier reports of transposon mutants of the closely related species *Mycobacterium marinum* and *Mycobacterium avium* harboring insertions in the orthologous gene whose ability to microaggregate and form biofilms were altered. Our findings point to an important role of SucT-mediated AG and LAM succinylation in modulating the cell surface properties of mycobacteria.

Covalent modification of cell envelope glycans with strategically placed discrete substituents such as various sugars, amino acids, phosphates, or acyl groups is a common strategy used by prokaryotes to adapt and survive under various stress conditions, including the environment of the infected host. Although not required for growth *per se*, these tailoring modifications may affect the biosynthesis, export, and/or biological activities of polysaccharides, thereby impacting the cell envelope composition and physical properties of the bacteria, their resistance to antimicrobials, and their interactions with host immune

responses (1–9). A classic example of how such modifications may thwart host defenses is masking by Gram-negative and Gram-positive bacteria of the negative charges conferred by the phosphate groups of lipopolysaccharide, wall teichoic acids, and lipoteichoic acids with positively charged amines under the form of amino sugars, phosphoethanolamine, or D-alanyl esters to evade killing by positively charged antimicrobial peptides. Although devoid of the canonical lipopolysaccharide and (lipo)teichoic acids, mycobacteria produce polysaccharides and lipopolysaccharides with unique structures that play critical roles in their cell envelope integrity and pathogenicity (10–11). The finding of covalent substituents modifying the structures of two of the dominant heteropolysaccharides produced by all *Mycobacterium* species, arabinogalactan (AG)<sup>2</sup> and lipoarabinomannan (LAM), suggests that mycobacteria have evolved similar strategies as other prokaryotes to promote their survival in different environments (12). To date, however, our understanding of the biological significance of these discrete substituents is, at best, rudimentary.

The arabinan domain of LAM is very similar to that of AG and made of stretches of  $\alpha$ -1,5–linked arabinofuranosyl (Araf) residues with precisely positioned  $\alpha$ -3,5 branch sites (Fig. 1). In *Mycobacterium tuberculosis* and a number of other slow- and fast-growing pathogenic mycobacteria (*Mycobacterium avium*, *Mycobacterium kansasii*, *Mycobacterium bovis*, *Mycobacterium leprae*, and *Mycobacterium abscessus*), the C2 position of a portion of the internal  $\alpha$ -3,5–branched Araf residues of the arabinan domain of AG may be modified with galactosamine substituents, a motif not found in LAM (13) (Fig. 1). Studies from our laboratory have shed light on the biosynthetic origin of this substituent (13) and begun to elucidate its contribution to modulation of the host immune response (14). Interestingly,

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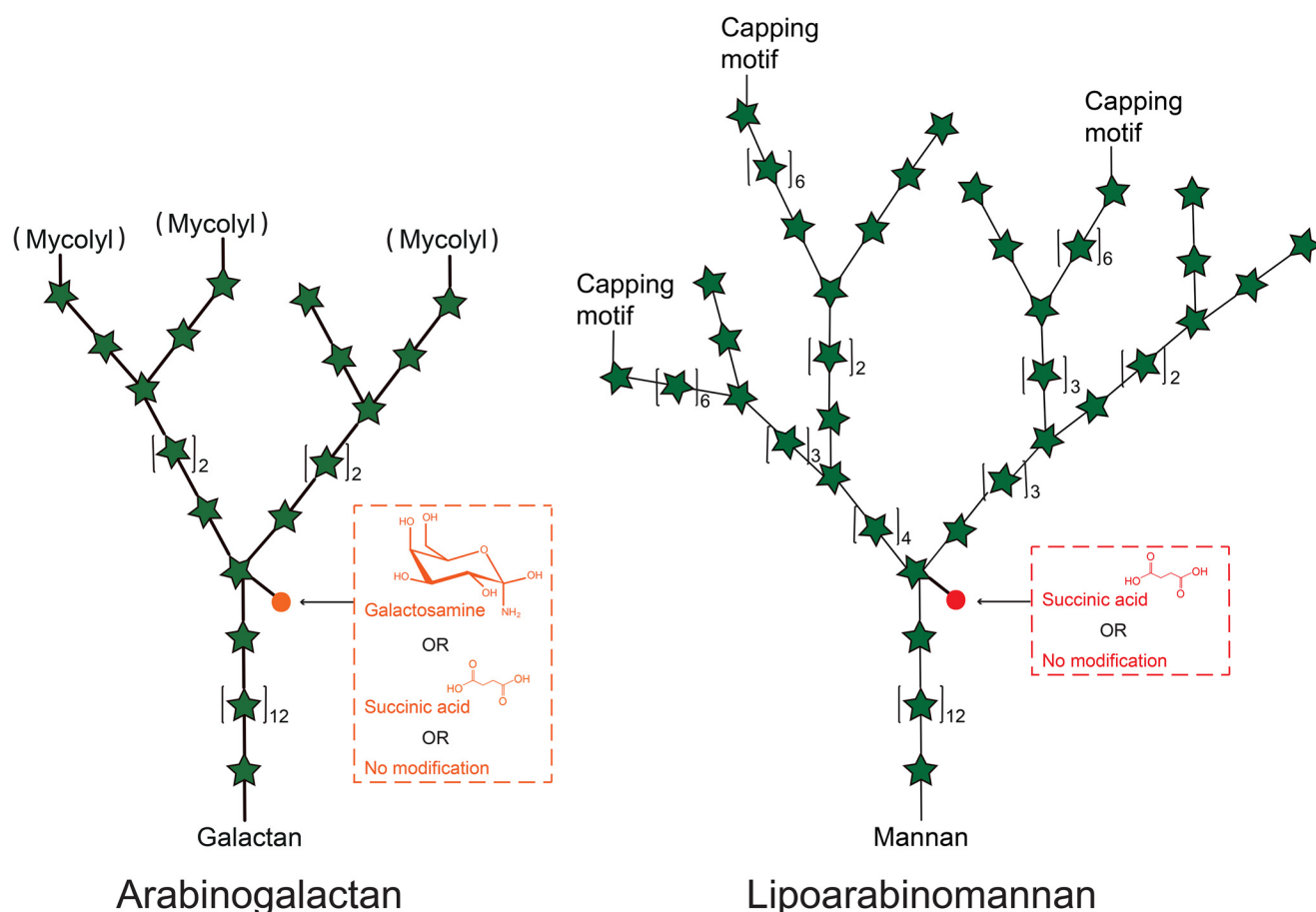
This article contains Figs. S1–S10, Tables S1–S4, and Supporting Methods.

<sup>1</sup> To whom correspondence should be addressed. Tel.: 970-491-3582; Fax: 970-491-1815; E-mail: Mary.Jackson@colostate.edu.

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<sup>2</sup> The abbreviations used are: AG, arabinogalactan; LAM, lipoarabinomannan; AM, D-arabino-D-mannan; LM, lipomannan; mAGP, mycolyl-AG-peptidoglycan; OADC, oleic acid–albumin–dextrose–catalase; ADC, albumin–dextrose–catalase; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl] glycine; AFM, atomic force microscopy; HMQC, heteronuclear multiple quantum coherence; TS, tryptic soy.

## Succinylation of mycobacterial arabinogalactan and LAM



**Figure 1. Detail of the chemical modifications affecting the internal arabinan domains of AG and LAM in *M. tuberculosis*.** Note that the succinylation of the mycolylated chains in arabinogalactan has been reported to be diminished or absent (15).

succinate groups were found to substitute the same positions of the arabinan domain as well as quantitatively minor  $\alpha$ -1,5-Araf positions of AG from *M. tuberculosis* and *Mycobacterium smegmatis* (15), the internal  $\alpha$ -3,5-branched Araf residues of the arabinan domain of LAM from *M. bovis* Bacillus Calmette-Guerin (BCG) (16), and possibly the same positions of LAM from *M. leprae* and *M. tuberculosis* (17–21). The presence of succinates has also been reported on the capsular polysaccharide D-arabino-D-mannan (AM) of *M. tuberculosis*, which shares with LAM a structurally identical arabinomannan domain (22). Succinates were finally found to substitute the C3 position of linear  $\alpha$ -1,5-Araf residues of LAM in *M. kansasii* (23). Collectively, these observations are suggestive of the widespread distribution of succinate motifs in the AG and LAM of mycobacteria, even though their precise position on these glycans may be variable from species to species. Succinyl substituents were estimated to occur at the level of about one to three motifs per arabinan chain in AG, one to six motifs per LAM molecule, and two to three motifs per AM molecule (15–17, 22). Neither the enzyme(s) catalyzing the introduction of these motifs in the arabinan domain of AG, AM, and LAM nor the precise biological significance of polysaccharide succinylation in mycobacteria are currently known, even though an association was suggested between the charge of various LAM isoforms (in part driven by succinate content) and their ability to stimulate CD1b-restricted T cells (19, 21). It has further been

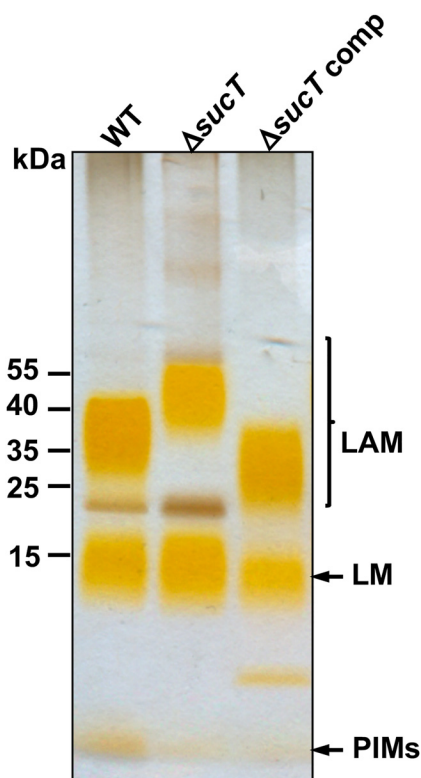
proposed that the negatively charged succinyl residues interact with the protonated galactosamine substituents, leading to a more rigid and tightened AG structure (15). Assuming that succinates are introduced during elongation of the arabinan domains of AG and LAM, these motifs could also serve as molecular signatures regulating elongation and branching (12). Alternatively, the apparent lack of succinylation on the mycolylated arabinan chains of AG (Fig. 1) has led to the hypothesis that succinylation negatively controls mycolylation (15).

Here we report the identification of a conserved mycobacterial acyltransferase, hereafter referred to as SucT, responsible for the succinylation of AG and LAM and the significant impact of disruption of *sucT* on the physiology of *M. smegmatis* cells. The possibility of generating mycobacterial mutants deficient in succinylation of cell envelope polysaccharides paves the way for studies aiming to determine the contribution of this discrete substituent to the physiology of slow- and fast-growing mycobacteria, their adaptation to the environment, and immunopathogenesis.

## Results

### Identification of a candidate enzyme for succinylation of AG and LAM

A bioinformatics search for protein candidates with potential for catalyzing the succinylation of the arabinan domains of AG



**Figure 2. Electrophoretic mobility of lipoglycans from WT *M. smegmatis* mc<sup>2</sup>155, the *sucT* mutant, and the complemented *sucT* mutant.** Lipoglycans extracted from WT mc<sup>2</sup>155, mc<sup>2</sup>Δ*sucT*, and mc<sup>2</sup>Δ*sucT*/pMVGHI-*sucT* (Δ*sucT* comp) were run on a 10–20% Tricine gel, followed by periodic acid-silver staining. PIMs, phosphatidylinositol mannosides.

and LAM identified five integral membrane proteins (Rv0111, Rv0228, Rv0517, Rv1254, and Rv1565c) in *M. tuberculosis* and nine homologous proteins in *M. smegmatis* (MSMEG\_0206, MSMEG\_0319, MSMEG\_0390, MSMEG\_2021, MSMEG\_3187, MSMEG\_3490, MSMEG\_5041, MSMEG\_5537, and MSMEG\_6230) showing sequence similarities with a family of trans-acylases (COG1835) involved in *O*-acylation of exported carbohydrate moieties in a variety of prokaryotes. Enzymes from this family include a number of *O* antigen, exopolysaccharide, and Nod factor acetyltransferases as well as macrolide acyltransferases (24). In line with the prototypical primary and secondary structures of these enzymes, all five *M. tuberculosis* candidate acyltransferases harbor nine to ten transmembrane segments and display conserved cytoplasmic and transmembrane amino acid residues reported to be critical for activity (24–26) (Fig. S1). All *M. tuberculosis* candidates are conserved in mycobacteria, with the exception of Rv0517, which is partially or completely deleted in some *M. tuberculosis* clinical isolates and is apparently missing from *M. leprae*. While TmaT (Rv0228 in *M. tuberculosis* and MSMEG\_0319 in *M. smegmatis*) was recently shown to acetylate mycolic acids (27–28), other candidates have not yet been functionally characterized.

Given the presence of succinate motifs on the AG and LAM of *M. smegmatis* (15, 17, 20), individual KO of each conserved candidate gene with clear orthologs in *M. tuberculosis* were generated by allelic replacement in this nonpathogenic, fast-growing model species (Fig. S2A), and their lipoglycans were analyzed by SDS-PAGE. As shown in Fig. 2 and Fig. S2B, the

MSMEG\_3187 mutant (orthologous to Rv1565c of *M. tuberculosis*) was the only one to present a lipoglycan profile significantly different from that of the WT parent strain. Although its lipomannan (LM) migrated similarly to that of the WT strain, its LAM migrated higher, suggestive of alterations in either size or charge (Fig. 2). Complementation of the MSMEG\_3187 KO with a replicative plasmid expressing a WT copy of the MSMEG\_3187 gene expressed from the *hsp60* promoter reversed the slow migration of LAM on SDS-PAGE beyond WT levels. MSMEG\_3187 shares 23% identity (40% similarity) on a 705-amino acid overlap with the *O* antigen acetyltransferase OafA from *Salmonella* (24). We renamed this protein SucT for the purpose of this study.

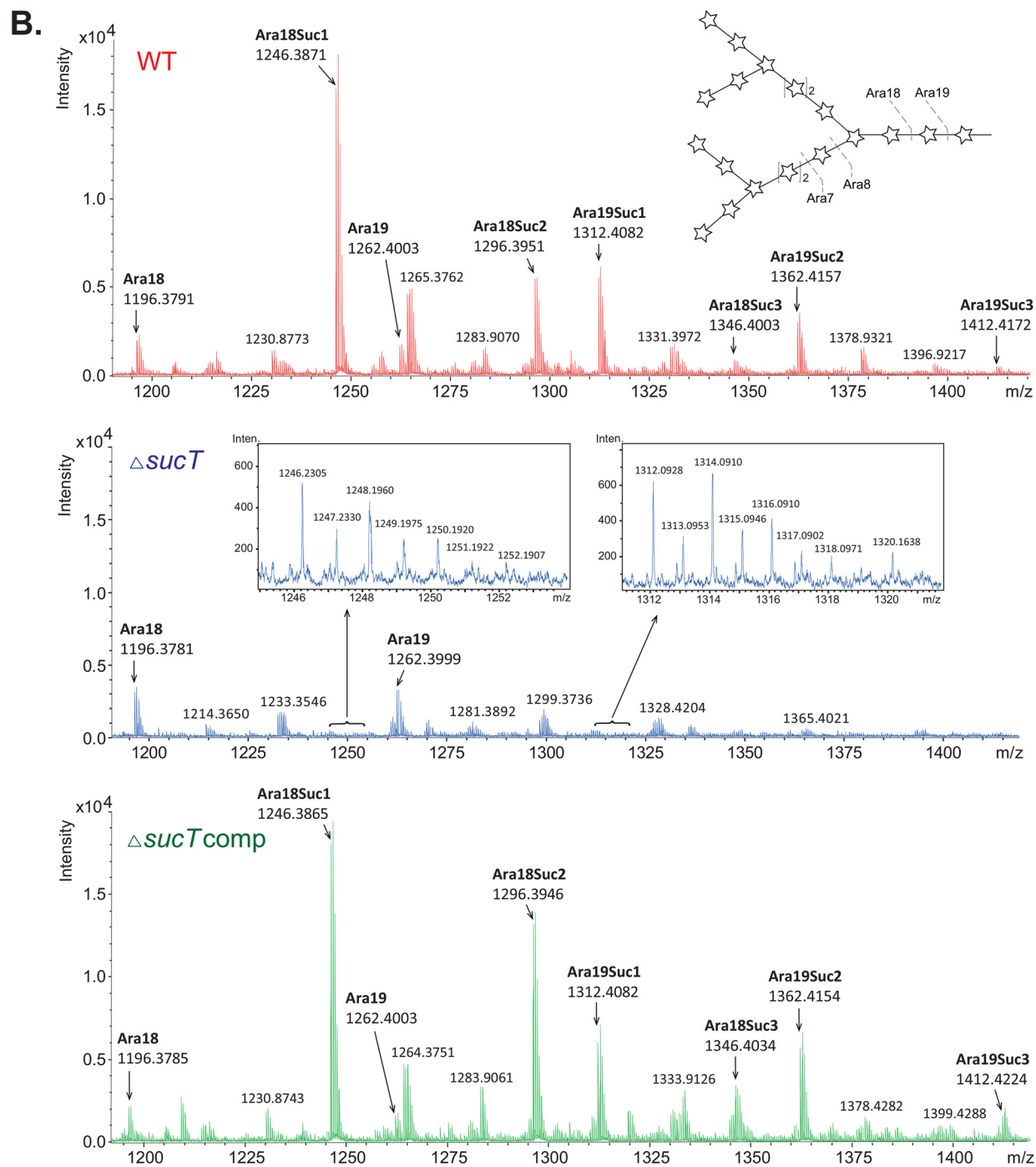
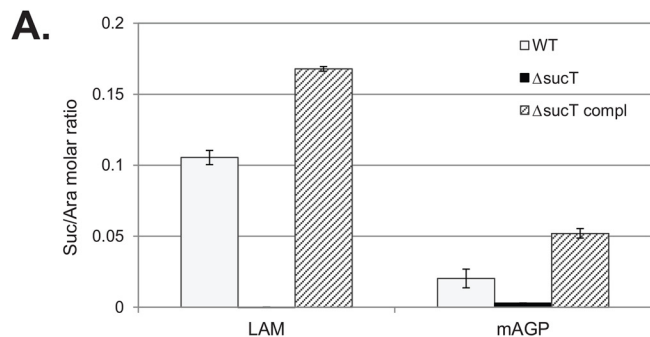
#### Disruption of *sucT* results in loss of succinyl substituents on the LAM of *M. smegmatis*

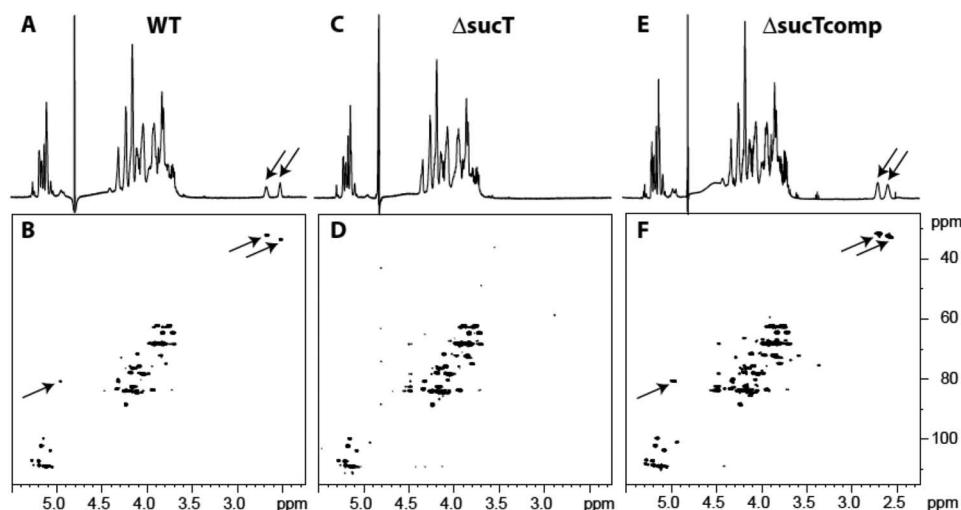
To determine whether a defect in succinylation and, thus, overall charge of the *sucT* mutant LAM might be responsible for its migration shift on SDS-PAGE, LAM was purified from the WT, *sucT* KO (*Msmg*Δ*sucT*), and complemented mutant strain (*Msmg*Δ*sucT*/pMVGHI-*sucT*) and submitted to a panel of analyses. Direct analysis of succinyl groups was performed by butanolysis of LAM prepared from the WT, mutant, and complemented mutant strains to yield dibutyl esters of any succinyl groups present (Supporting Methods). GC/MS analysis revealed a complete absence of succinates in the mutant LAM. LAM succinylation was restored beyond WT levels in the complemented mutant. Quantitation of the ratio of succinyl to arabinosyl groups showed a ratio of 1:9.5 (S.D. ± 0.4 for two determinations) for the WT strain and 1:6 (S.D. ± 0.1 for three determinations) following ectopic overexpression of MSMEG\_3187 in *Msmg*Δ*sucT*/pMVGHI-*sucT* (Fig. 3A).

Next, LAM prepared from each of the three strains was analyzed for the presence of succinates by 1D and 2D NMR spectroscopy (16). The 1D <sup>1</sup>H spectrum of LAM prepared from the WT strain showed the characteristic two pseudotriplets (*J* = 6.5 Hz) of similar intensities at 2.50 and 2.66 ppm (Fig. 4A) assigned to methylene groups of succinyl units. Their corresponding carbons were characterized at 34.7 and 33.3 ppm, respectively, on the 2D <sup>1</sup>H-<sup>13</sup>C HMQC spectrum (Fig. 4B). The cross-peaks between <sup>1</sup>H δ 4.96/<sup>13</sup>C δ 81.9 and <sup>1</sup>H δ 4.94/<sup>13</sup>C δ 82.1 further typify the presence of succinyl residues on the C2 of α-3,5-branched Araf residues and, possibly, some adjacent α-1,5-Araf residues (16). These signals were absent from the 1D <sup>1</sup>H and 2D HMQC spectra of the mutant LAM (Fig. 4, C and D) and restored upon genetic complementation Fig. 4, E and F). Collectively, our analyses support the conclusions that succinates substitute α-3,5-branched Araf residues of the arabinan domain of LAM in *M. smegmatis*, as reported previously in *M. bovis* BCG (16), and that the *M. smegmatis* *sucT* KO is devoid of any such substituents.

Because of the report of succinates on the LM of an unspecified *M. smegmatis* strain (20), we next sought to verify whether the succinylation defect extended to the mutant's LM. Comparison of the WT and mutant LM by NMR spectroscopy, however, failed to identify any succinates on the LM of the *M. smegmatis* mc<sup>2</sup>155 parent strain used in this study (Fig. S3). Overall,

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**Figure 4. NMR analysis of LAM prepared from the WT, mutant, and complemented mutant strains.** Shown are 1D  $^1\text{H}$  (A, C, and E) and 2D  $^1\text{H}$ - $^{13}\text{C}$  (B, D, and F) HMQC NMR spectra. Arrows point to the signals typifying succinate detection and localization (see text for details).

no significant structural differences were noted between the LM prepared from *MsmgΔsucT* and the WT strain.

Interestingly, a screen for *Mycobacterium marinum* transposon mutants devoid of mannose caps on LAM yielded a strain harboring a transposon insertion in *MMAR\_2380*, the ortholog of *sucT* in this species (75% identity/85% similarity at the protein level with MSMEG\_3187) (29). Not only was this mutant confirmed to lack mannoside caps on LAM, it also displayed a higher degree of branching of both the mannan core and the arabinan domain compared with WT LAM and decreased acylation of the phosphatidylinositol anchor of both LM and LAM. In contrast to the *M. marinum* *MMAR\_2380* mutant, *MsmgΔsucT* failed to show any reduction in the incorporation of [1,2- $^{14}\text{C}$ ]acetate into the acyl chains of LM and LAM (Fig. S4A). Likewise, quantitative analyses of the alditol acetate and per-*O*-methylated alditol acetate derivatives of the WT, mutant, and complemented mutant LAM did not point to any significant alterations in the *Ara*<sub>f</sub> to *Man*<sub>p</sub> ratio of the mutant LAM (Table S1) or increase in the degree of branching of its mannan and arabinan domains (Table S2). The relative proportion of *Ara*<sub>4</sub> to *Ara*<sub>6</sub> arabinan termini of LAM was also similar in the WT and mutant strains (relative percentage of *Ara*<sub>2</sub>, *Ara*<sub>4</sub>, and *Ara*<sub>6</sub> oligoarabinosides released upon *Cellulomonas gelida* endoarabinanase digestion:  $58.7\% \pm 4.6\%$ ,  $37.5\% \pm 5.2\%$ , and  $3.8\% \pm 0.7\%$ , respectively, in the WT strain compared with  $60.9\% \pm 3.4\%$ ,  $34.3\% \pm 4.3\%$ , and  $4.8\% \pm 1.0\%$  in the *sucT* mutant; average  $\pm$  S.D. of three technical replicates).  $^{31}\text{P}$  NMR and LC/MS analysis of the oligoarabinosides released upon *C. gelida* endoarabinanase digestion of deacylated LAM further confirmed the presence of the expected phosphoinositol capping motifs of *M. smegmatis* LAM in the WT and mutant strains (Fig. S5, A and B). Metabolic labeling of WT and mutant cultures with [ $^{14}\text{C}$ ]glucose finally pointed to similar rates of *de*

*novo* lipoglycan synthesis in both strains (Fig. S4B). Collectively, these results suggest that, besides abolishing succinylation, the disruption of *sucT* did not appreciably affect, either qualitatively or quantitatively, the biosynthesis of LAM in *M. smegmatis*.

#### Effect of inactivating *sucT* on the succinylation of *M. smegmatis* AG

Given the structural similarity of the arabinan domains of AG and LAM, we next sought to determine whether the disruption of *sucT* had any impact on the succinate content of AG from *M. smegmatis*. To this end, the mycolyl-AG-peptidoglycan (mAGP) complex prepared from the WT, mutant, and complemented mutant strains was subjected to the same butanolysis procedure as for LAM, and succinyl substituents as their dibutyl esters were analyzed by GC/MS. Compared with the WT parent, mAGP from the *sucT* KO showed an 84.7% decrease in succinate content. Quantitation of the ratio of succinyl to arabinosyl groups yielded a ratio of 1:52 (S.D.  $\pm$  14.3 for three determinations) for the WT strain, 1:350 (S.D.  $\pm$  20.9 for two determinations) for *MsmgΔsucT*, and 1:19 (S.D.  $\pm$  1.2 for three determinations) for *MsmgΔsucT*/pMVGH1-*sucT* (Fig. 3A). To gain further insight into the degree of succinylation of AG in the different strains, their mAGP was digested with endogenous *M. smegmatis* endoarabinanase, and the released oligoarabinosides were analyzed by LC/MS as described in the Supporting Methods. The mass spectra of the arabinans released from the WT and complemented mutant AG revealed nonsuccinylated and succinylated *Ara*<sub>18</sub> and *Ara*<sub>19</sub> oligoarabinosides, whereas only the nonsuccinylated variants were detected in *MsmgΔsucT* (Fig. 3B). Similar to the situation with *M. tuberculosis* (15), the fact that *Ara*<sub>7</sub> fragments showed a significantly ( $\sim$  3-fold) smaller degree of succinylation is inter-

**Figure 3. Succinate content of AG and LAM prepared from WT *M. smegmatis* mc<sup>2</sup>155, the  $\Delta$ *sucT* mutant, and the complemented mutant strain.** A, the amounts of succinates and arabinose residues in the same LAM and mAGP samples prepared from the WT, mutant, and complemented mutant ( $\Delta$ *sucT* *compl*) strains were quantified as described in the Supporting Methods. Results are expressed as average  $\pm$  S.D. succinate/arabinose molar ratios from three technical replicates. B, endogenous endoarabinanase digestion of mAGP from the WT, mutant, and complemented mutant strains. Analysis of the products of the reaction by QTOF LC/MS in the negative ion mode revealed characteristic [M-2H] $^{-2}$  ions corresponding to oligoarabinosides with succinyl groups in the WT and complemented mutant that are not found in the *sucT* mutant mAGP.

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preted as the succinyl residues being located on the interior branched  $\alpha$ -3,5-Araf residue. This observation, combined with the fact that Ara<sub>18</sub> may have up to four succinyl substituents, leads to the identification of quantitatively minor positions of succinylation on adjacent linear  $\alpha$ -1,5-branched Araf residues (Fig. 3B). The fact that *Msmg* $\Delta$ *sucT* mAGP was not entirely devoid of succinates, according to the butanolysis analysis, could indicate that succinyl groups substitute, in a SucT-independent manner, other positions of AG not revealed by our LC/MS analysis of oligoarabinosides. Alternatively, because the GC/MS analysis of succinyl substituents was performed on mAGP rather than on purified AG, we cannot exclude that the remaining succinates arose from peptidoglycan and/or mycolic acids.

Analyses of the monosaccharide composition (Table S3) and glycosyl linkages (Table S4) of the WT, mutant, and complemented mutant AG otherwise failed to reveal any significant structural alterations in the galactan or arabinan domains of the mutant AG. The degree of mycolylation of the mutant AG was also similar to that measured in the WT strain (Table S3).

### Alterations in the cell surface properties of the *sucT* mutant

Two previous studies have reported on the isolation and partial characterization of *Mycobacterium* mutants carrying transposon insertions in *sucT* orthologs. First was the study by Driesen *et al.* (29), mentioned above, in which a *M. marinum* *sucT* KO mutant was identified as part of a screen for strains devoid of mannoside caps on LAM. Second was the screening by Yamazaki *et al.* (30) of an *M. avium* transposon mutant library for strains impaired in biofilm formation, which also yielded a mutant harboring an insertion in *sucT*. Although neither of these studies reported on the function of *sucT* and its impact on succinylation of AG and LAM, interesting observations were made regarding the growth and cell surface properties of the mutants. The *M. marinum* mutant, although displaying WT colony morphology on 7H10 agar plates, was reported to be hyperaggregative in 7H9-ADC-Tween 80 medium (29). The *M. avium* *sucT* mutant, on the other hand, was found to display an altered colony morphology on agar and to be impaired in its ability to form biofilms on polyvinyl chloride microplates (30). Our observations point to *Msmg* $\Delta$ *sucT* sharing characteristics of both the *M. marinum* and *M. avium* *sucT* transposon mutants in that it aggregated in 7H9-ADC-Tween 80 medium and displayed an altered colony morphology on 7H11-OADC agar (Fig. 5A). Monitoring of the absorbance and colony-forming unit counts of *Msmg* $\Delta$ *sucT* cultures over time in 7H9-ADC-tyloxapol medium (where satisfactory bacterial dispersion was achieved) also reproducibly pointed to a slight reduction in growth of the mutant compared with the WT strain, marked by an extended lag period (Fig. S6A). Despite having recovered the ability to succinylate both AG and LAM and a WT colonial morphology (Fig. 5A), the complemented mutant showed a growth rate more comparable with that of the *sucT* KO (Fig. S6A), a result that may tentatively be explained by the hyper-succinylation of AG and LAM in this strain. Further analysis revealed that *Msmg* $\Delta$ *sucT* was slightly retarded in its ability to form surface biofilm pellicles in Sauton's medium, a phenotype probably resulting (at least in part) from its slower growth. Sur-

face pellicle formation was partially restored upon genetic complementation (Fig. S6B).

Because the hyperaggregative properties and biofilm defect of the *M. smegmatis* *sucT* KO were suggestive of changes in cell surface hydrophobicity, the WT, mutant, and complemented mutant were next compared for their ability to bind the lipophilic dye Congo red (31). In liquid TS broth, *Msmg* $\Delta$ *sucT* cells bound more than four times more Congo red than the WT and complemented strains, pointing to a clear increase in cell surface hydrophobicity (Fig. 5B). This phenotype also reflected on TS agar supplemented with Congo red, where the WT and complemented mutant strains grew as dry white colonies, whereas the *sucT* KO grew as glossy red colonies (Fig. 5B). Drug susceptibility testing further indicated that the *sucT* mutant was eight times more susceptible to rifampicin, a drug thought to penetrate the *M. smegmatis* outer membrane through passive diffusion (Table 1).

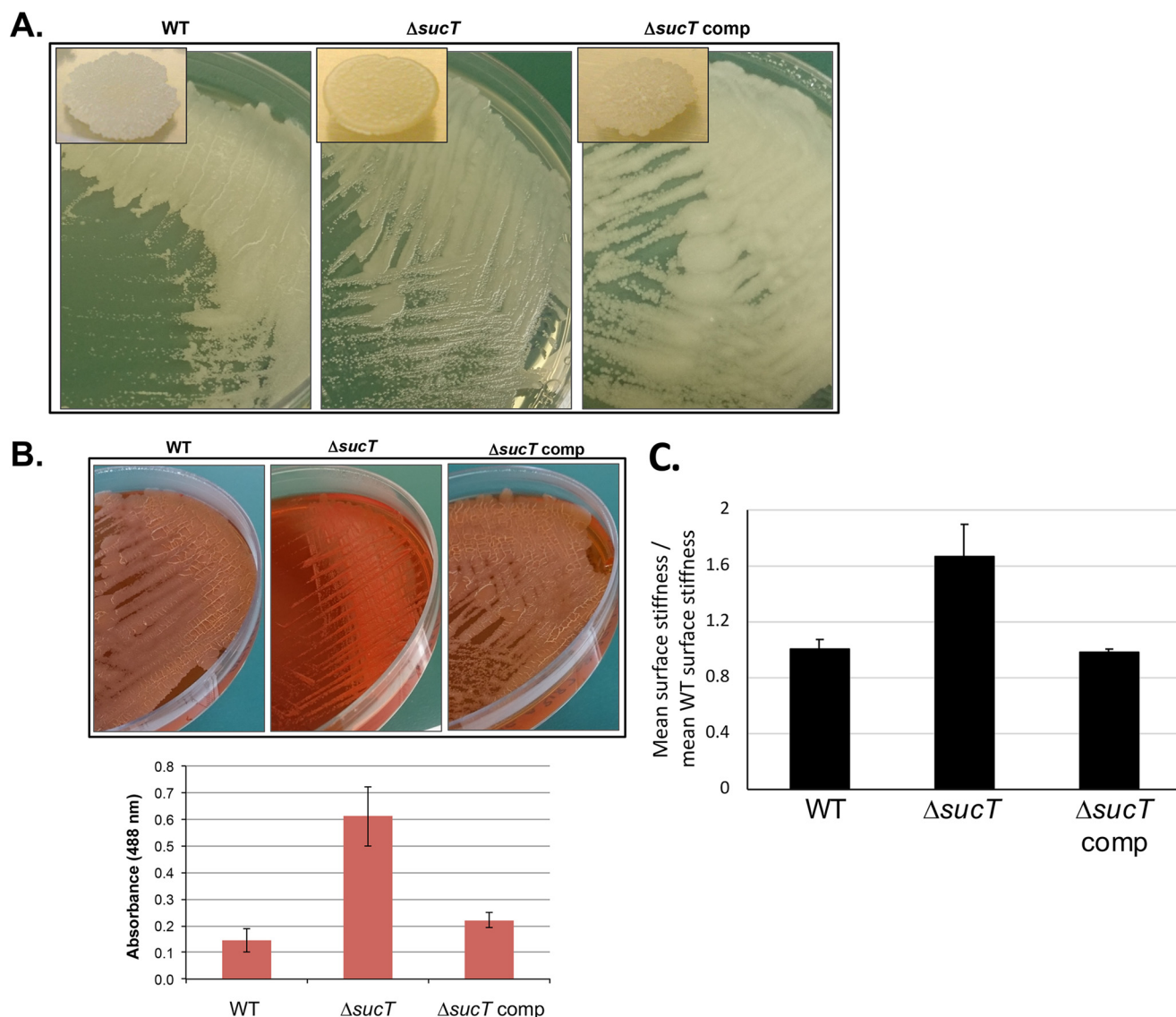
To gain further insight into the cell envelope properties of the *sucT* mutant, the surface rigidity of the WT, mutant, and complemented mutant cells in relation to their height was measured using correlated optical fluorescence microscopy and atomic force microscopy. Results pointed to an  $\sim$ 1.7-fold increase in the mean surface rigidity of the mutant compared with the WT and complemented mutant strains (Fig. 5C).

To investigate the possible reasons underlying the increased hydrophobicity and other surface alterations of the *M. smegmatis* *sucT* mutant, the surface lipids from this strain were finally extracted and compared with the cell surface lipid contents of the WT and complemented mutant. TLC analyses pointed to the reproducible buildup of three compounds in the *sucT* mutant that were either not detected or present in much lesser quantities at the surface of the WT and complemented strains (Fig. S7A). The nature of these products is at present not known, but staining with  $\alpha$ -naphthol suggests that they may be glycolipids. TLC analyses of total lipids from the same *M. smegmatis* strains upon metabolic labeling with [1,2-<sup>14</sup>C]acetate did not reveal any other notable differences between the WT and *sucT* KO (Fig. S7B). Likewise, comparative analysis by LC/MS of the contents of the WT and *sucT* mutant in another succinylated lipopolysaccharide, known as the methylglucose lipopolysaccharide, failed to reveal any significant differences between the two strains (Fig. S8).

## Discussion

Similar to other mycobacterial species, *M. smegmatis* has been known to acylate its two major cell envelope glycans, AG and LAM, with succinyl groups even though their biological significance and biosynthetic origin have remained elusive. The data presented here show that succinyl substituents precisely modify the C2 position of  $\alpha$ -3,5-branched Araf residues of the arabinan domain of LAM in *M. smegmatis* and possibly the same position of the arabinan domain of AG, with the possibility for adjacent linear  $\alpha$ -1,5-branched Araf residues to be succinylated as well. Data further show that SucT (MSMEG\_3187) is the sole enzyme responsible for this modification in both LAM and AG.

The identification of SucT as the enzyme responsible for the modification of AG and LAM with succinyl groups raises ques-



**Figure 5. Alterations in the cell surface properties of the *M. smegmatis* *sucT* mutant.** A, colony morphology of WT  $mc^2$ 155,  $mc^2\Delta sucT$ , and  $mc^2\Delta sucT/pMVGH1-sucT$  ( $\Delta sucT$  comp) after 3 days of incubation on 7H11-OADC agar at 37 °C. B, Congo red binding on a TS agar plate and in TS liquid medium (graph). Shown on the graph are the average  $\pm$  S.D. absorbances of acetone extracts measured for three biological replicates. C, analysis of the cell surface rigidity of the WT, mutant, and complemented mutant strains by correlated optical fluorescence and AFM. Two-sided rank sum test demonstrates a statistically significant difference in stiffness between the *sucT* mutant and both the WT ( $p = 0.0214$ ) and the *sucT* complemented mutant ( $p = 0.0011$ ).

**Table 1**  
Susceptibility of the *M. smegmatis* *sucT* knockout mutant to antibiotics

MIC were determined in 7H9-ADC-tyloxapol and MIC values are in micrograms per milliliter. MIC determinations were performed two to three times on independent culture batches. HYG, hygromycin; KAN, kanamycin; GEN, gentamycin; CIP, ciprofloxacin; INH, isoniazid; EMB, ethambutol; RIF, rifampicin; ND, not determined.

Strains	HYG	KAN	GEN	RIF	INH	CIP	EMB
WT	10	1.2	2.5	50	12.5	0.3	0.8
$mc^2\Delta sucT$	5–10	1.2	2.5	6.2	12.5	0.15	0.8–1.6
$mc^2\Delta sucT/pMVGH1-sucT$	ND	0.6	ND	50	25	0.3	0.8

tions regarding where and when the introduction of these substituents occurs. Both the arabinan domains of AG and LAM are synthesized on the periplasmic face of the plasma membrane through what appears to be sequential addition of AraF residues by polyprenyl-phosphate-arabinose-dependent arabinosyltransferases (10). It is thus reasonable to assume that the succinylation of AG and LAM takes place on the periplasmic side of the membrane. The mechanism of acyl transfer catalyzed by the family of integral membrane trans-acylases to

which SucT belongs, however, has to date not been fully resolved. The fact that the repeating unit of O-antigen from *Salmonella* Typhimurium is synthesized intracellularly originally led Slauch *et al.* (24) to predict that OafA-like enzymes catalyzed acyl transfer on the cytoplasmic face of the plasma membrane using acyl-CoAs as acyl donors. The rather widespread distribution of functional residues across cytoplasmic loops and transmembrane segments of these enzymes (25–26), however, makes it difficult to precisely map their catalytic site,



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raising the possibility that acyl transfer may perhaps occur on the periplasmic side from still unknown high-energy acyl donors. The cytoplasmic succinylation of Araf residues in mycobacteria would entail modification of Araf on the cytoplasmic arabinose donor, polyprenyl-phosphate-arabinose. In light of our failure to detect succinylated polyprenyl-phosphate-arabinose by LC/MS in total lipid fractions from *M. tuberculosis* (data not shown), the hypothesis of periplasmic modification of the arabinan domains of AG and LAM is preferred.

A second important question regarding the catalytic reaction catalyzed by SucT relates to the precise stage in the biosynthesis of AG and LAM at which succinylation occurs. The modification of Araf residues during elongation of the arabinan of AG/LAM could suggest a role of these substituents in governing the elongation and branching of the arabinan domain. Succinylation of AG and LAM upon completion of their synthesis, on the contrary, would rule out their involvement in biosynthesis and rather point to other functions in the physiology of the bacterium and/or its interaction with the host. The conflicting phenotypes of the *M. marinum* and *M. smegmatis* *sucT* mutants, where the first one displays a significantly altered LAM structure (29) and the second does not, preclude a conclusive answer to this question. It is clear from our results that succinylation is neither critical for biosynthesis of the galactan, mannan, and arabinan domains of AG and LAM nor for the capping of LAM with phosphoinositol caps in *M. smegmatis*. As a first step toward determining whether differences in the LAM biosynthetic pathways of slow- and fast-growing mycobacteria might have accounted for the contrasting phenotypes between the LAM structures of the *M. marinum* and *M. smegmatis* *sucT* mutants, we tested the ability of the *sucT* gene from *M. tuberculosis* (*Rv1565c*; 83% identity at the amino acid level with MMAR\_2380 from *M. marinum* and 75% identity with MSMEG\_3187 from *M. smegmatis*) to complement the *M. smegmatis* *sucT* mutant. The results, which are presented in Fig. S9, point to partial restoration of LAM succinylation in the complemented strain, validating *Rv1565c* as a LAM succinyltransferase. The reason underlying the more critical role of succinylation in elongation and branching of LAM (and perhaps AG) in slow- compared with fast-growing *Mycobacterium* species thus remains to be determined. Unfortunately, our attempts to develop a cell-free assay to determine whether *M. smegmatis* SucT acts on the arabinan domain during its elongation or upon its completion failed to detect any succinyltransferase activity associated with SucT, whatever the enzyme source (*Escherichia coli* lysates expressing *M. smegmatis* *sucT*, cell-free extracts prepared from *M. smegmatis* WT and the *sucT* KO) or acceptor substrates used: neoglycolipid acceptors mimicking the interior linear and branched structures of the arabinan domain of AG and LAM (Fig. S10) or intact mutant LAM devoid of succinyl substituents (data not shown).

A clear conserved effect of LAM and/or AG succinylation across slow- and fast-growing *Mycobacterium* species, based on reports of *sucT* mutants in *M. avium*, *M. marinum*, and now *M. smegmatis*, is on the cell surface properties of the bacilli (29–30). That these changes in surface properties are the result of alterations in the mycolylation of AG, as proposed earlier

(15), was precluded by our analyses of the *M. smegmatis* mutant. Instead, our analyses of the cell surface composition of *MsmgΔsucT* point to an impact of AG and/or LAM succinylation on the production and/or export of some still unknown lipids. The control AG and LAM succinylation has on the bacilli's surface rigidity and hydrophobicity likely has significant implications for the way mycobacteria interact with their environment, including that of the infected host. A defect in biofilm formation, for instance, is expected to negatively impact the ability of mycobacteria to colonize environmental and host substrata as well as their tolerance to biocides. From a virulence standpoint, the *M. avium* *sucT* KO has been reported to be impaired in its ability to invade bronchial epithelial cells and cause lung infection in mice (32) while replicating similarly as its WT parent in THP-1 human mononuclear phagocytes (33). Whether AG/LAM succinylation contributes similarly to the adaptation of tuberculous mycobacteria to their environment and to immunopathogenesis is currently under investigation in our laboratory.

## Experimental procedures

### Bacterial strains and growth conditions

*M. smegmatis* mc<sup>2</sup>155 was grown under agitation at 37 °C in Middlebrook 7H9 medium supplemented with 10% albumin–dextrose–catalase (ADC) (BD Biosciences) and 0.05% Tween 80 or 0.05% tyloxapol, on Middlebrook 7H11 agar supplemented with 10% oleic acid–albumin–dextrose–catalase (OADC) (BD Biosciences), in glycerol–alanine–salts (GAS) medium with 0.05% tyloxapol, or in Sauton's medium as surface pellicles. Apramycin, kanamycin, hygromycin, and streptomycin were added to final concentrations of 25 μg ml<sup>-1</sup>, 25 μg ml<sup>-1</sup>, 50 μg ml<sup>-1</sup>, and 20 μg ml<sup>-1</sup>, respectively.

### *M. smegmatis* knockout and complemented knockout mutants

The temperature-sensitive/*sacB* method was used to knock out each of the candidate succinyltransferases of *M. smegmatis* (34). Allelic exchange substrates consisted of an antibiotic cassette (apramycin, streptomycin, kanamycin, or hygromycin) flanked by ~1,000 bp of upstream and downstream homologous DNA sequence flanking MSMEG\_2021, MSMEG\_3187, MSMEG\_5041, MSMEG\_5537, and MSMEG\_6230. Primer sequences for these constructs are available upon request. KO mutants were confirmed by PCR using sets of primers located outside of the allelic exchange substrates. For complementation of the MSMEG\_3187 KO, the entire coding sequence of MSMEG\_3187 was PCR-amplified from *M. smegmatis* mc<sup>2</sup>155 genomic DNA and cloned into the replicative expression plasmid pMVGH1 (35), yielding pMVGH1-*sucT*. pMVGH1-*Rv1565c*, the plasmid used to complement the MSMEG\_3187 KO with the *Rv1565c* gene from *M. tuberculosis*, was PCR-amplified from *M. tuberculosis* H37Rv genomic DNA and similarly cloned into pMVGH1.

### Metabolic labeling

Radiolabeling of *M. smegmatis* with [1,2-<sup>14</sup>C]acetic acid (1 μCi ml<sup>-1</sup>; specific activity, 52 mCi mmol<sup>-1</sup>; PerkinElmer Life

Sciences) or [ $^{14}\text{C}$ ]glucose (1  $\mu\text{Ci ml}^{-1}$ ; specific activity, 5 mCi  $\text{mmol}^{-1}$ ; American Radiolabeled Chemicals) was performed in 7H9-ADC-tyloxapol and GAS-tyloxapol, respectively, at 37 °C for 4 h with shaking.

#### Preparation and analysis of lipids, methylglucose lipopolysaccharides, lipoglycans, and arabinogalactan

Total lipids from *M. smegmatis* cells were extracted with  $\text{CHCl}_3/\text{CH}_3\text{OH}$  (1:2, v/v) for 2 h at 56 °C, followed by two 2-h extractions with  $\text{CHCl}_3/\text{CH}_3\text{OH}$  (2:1, v/v) at 56 °C. Surface-exposed lipids were extracted from whole cells in water-saturated 1-butanol as described previously (36), and the butanol-treated cells were then re-extracted twice for 2 h with  $\text{CHCl}_3/\text{CH}_3\text{OH}$  (2:1, v/v) at 56 °C to recover all remaining extractable lipids. Cold and radiolabeled lipids were analyzed on silica gel 60–precoated TLC plates ( $F_{254}$ , Merck) in a variety of solvent systems to resolve lipids of various polarities. TLC plates were revealed by spraying with cupric sulfate (10% w/v in an 8% v/v phosphoric acid solution) or  $\alpha$ -naphthol (1% w/v in ethanol) and charring. Radiolabeled products were visualized using a Sapphire Biomolecular Imager (Azure Biosystems).

Methylglucose polysaccharides were extracted from whole cells as described previously (37) and analyzed as described by De *et al.* (38) by ultraperformance LC on a Waters Acquity UPLC H-Class system coupled to a Bruker Maxis Plus quadrupole time-of-flight MS instrument.

Lipoglycan and mAGP complex extraction from delipidated cells followed earlier procedures (15, 39). Lipoglycans were purified by gel exclusion chromatography (40) and analyzed by SDS-PAGE on commercial Novex<sup>TM</sup> 10–20% Tricine gels stained with periodic acid–Schiff reagent. Other procedures related to the structural analysis of LAM and mAGP are detailed in the Supporting Methods.

#### Congo red binding

*M. smegmatis* strains were tested for Congo red binding in TS broth as described by Etienne *et al.* (31). Briefly, the strains were cultivated for 3 days at 37 °C with shaking in TS broth containing 100  $\mu\text{g/ml}$  Congo red. Cells were collected by centrifugation and washed extensively with distilled water until the supernatant was colorless. Cells were next resuspended in 1 ml of acetone, vortexed, and gently shaken for 2 h at room temperature prior to pelleting by centrifugation. Congo red in the supernatants was quantified spectrophotometrically at 488 nm. Congo red binding was defined as the  $A_{488 \text{ nm}}$  of the acetone extracts divided by the dry weight (in milligrams) of the cell pellet.

#### Drug susceptibility testing

Minimum inhibitory concentration (MIC) values were determined in 7H9-ADC-tyloxapol in a total volume of 100  $\mu\text{l}$  in 96-well microtiter plates. *M. smegmatis* cultures grown to early log phase ( $A_{600 \text{ nm}} \sim 0.2$ ) were diluted to a final concentration of  $10^6$  cfu/ml and incubated in the presence of serial dilutions of the drugs for 2 days at 37 °C. MICs were determined using the resazurin blue test (41).

#### Correlated optical fluorescence and atomic force microscopy

Preparation conditions and the technical setup for AFM experiments were conducted according to Eskandarian *et al.* (42). Cells of the *M. smegmatis* WT expressing Wag31-GFP were mixed with nonfluorescent *Msmg* $\Delta$ *sucT* and deposited on a polydimethylsiloxane-coated coverslip. WT cells were distinguished from *Msmg* $\Delta$ *sucT* cells by optical fluorescence microscopy. AFM measurements were made using a Dimension Icon scan head (Bruker) using ScanAsyst fluid cantilevers (Bruker) with a nominal spring constant of  $0.7 \text{ N m}^{-1}$  in Peak Force quantitative nanomechanical mode at a force setpoint of  $\sim 1 \text{ nN}$  and typical scan rates of 0.3 Hz. Indentation on the cell surface was estimated to be  $\sim 10 \text{ nm}$  with a range of  $\sim 5 \text{ nm}$  in the  $z$  axis. Height, peak force error, and Derjaguin-Muller-Toporov (DMT) modulus channels were recorded for all scanned images in the trace and retrace directions. Images were processed using Gwyddion (Department of Nanometrology, Czech Metrology Institute; <http://gwyddion.net>).<sup>3</sup> ImageJ was used for extracting bacterial cell profiles from height and DMT modulus images in a tabular format. A two-sided Wilcoxon rank-sum  $U$  test was used to analyze the data, with a continuity correction and confidence level of 95% using MatLab. AFM raw data are available at <https://figshare.com/s/9dac3d318fceb04653ca>.<sup>3</sup>

*Author contributions*—Z. P., S. K. A., J. M. B., M. Joe, R. B., J. N., T. L. L., M. G., M. M., and M. Jackson conceptualization; Z. P., S. K. A., J. M. B., H. A. E., M. Joe, R. B., C. R., J. N., T. L. L., and M. G. investigation; Z. P., S. K. A., J. M. B., H. A. E., M. Joe, R. B., C. R., V. J., J. N., T. L. L., M. G., and M. M. methodology; Z. P., J. M. B., H. A. E., C. R., T. L. L., M. G., M. M., and M. Jackson writing-original draft; Z. P., J. M. B., H. A. E., J. N., T. L. L., M. G., M. M., and M. Jackson writing-review and editing; S. K. A., J. M. B., H. A. E., M. G., M. M., and M. Jackson formal analysis; T. L. L. and M. Jackson funding acquisition; M. M. and M. Jackson supervision; M. Jackson project administration.

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#### References

1. Raetz, C. R., Reynolds, C. M., Trent, M. S., and Bishop, R. E. (2007) Lipid A modification systems in Gram-negative bacteria. *Annu. Rev. Biochem.* **76**, 295–329 [CrossRef Medline](#)
2. Swoboda, J. G., Campbell, J., Meredith, T. C., and Walker, S. (2010) Wall teichoic acid function, biosynthesis, and inhibition. *ChemBioChem* **11**, 35–45 [Medline](#)
3. Brown, S., Xia, G., Luhachack, L. G., Campbell, J., Meredith, T. C., Chen, C., Winstel, V., Gekeler, C., Irazoqui, J. E., Peschel, A., and Walker, S. (2012) Methicillin resistance in *Staphylococcus aureus* requires glycosylated wall teichoic acids. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 18909–18914 [CrossRef Medline](#)
4. Needham, B. D., and Trent, M. S. (2013) Fortifying the barrier: the impact of lipid A remodeling on bacterial pathogenesis. *Nat. Rev. Microbiol.* **11**, 467–481 [CrossRef Medline](#)

<sup>3</sup> Please note that the JBC is not responsible for the long-term archiving and maintenance of this site or any other third party-hosted site.

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- Whitfield, C., and Trent, M. S. (2014) Biosynthesis and export of bacterial lipopolysaccharides. *Annu. Rev. Biochem.* **83**, 99–128 [CrossRef Medline](#)
- Schneewind, O., and Missiakos, D. (2014) Lipoteichoic acids, phosphate-containing polymers in the envelope of gram-positive bacteria. *J. Bacteriol.* **196**, 1133–1142 [CrossRef Medline](#)
- Maldonado, R. F., Sá-Correia, I., and Valvano, M. A. (2016) Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. *FEMS Microbiol. Rev.* **40**, 480–493 [CrossRef Medline](#)
- Rajagopal, M., and Walker, S. (2017) Envelope structures of Gram-positive bacteria. *Curr. Top. Microbiol. Immunol.* **404**, 1–44 [Medline](#)
- Gerlach, D., Guo, Y., De Castro, C., Kim, S. H., Schlatterer, K., Xu, F. F., Pereira, C., Seeberger, P. H., Ali, S., Codée, J., Sirisarn, W., Schulte, B., Wolz, C., Larsen, J., Molinaro, A., et al. (2018) Methicillin-resistant *Staphylococcus aureus* alters cell wall glycosylation to evade immunity. *Nature* **563**, 705–709 [CrossRef Medline](#)
- Angala, S. K., Belardinelli, J. M., Huc-Claustre, E., Wheat, W. H., and Jackson, M. (2014) The cell envelope glycoconjugates of *Mycobacterium tuberculosis*. *Crit. Rev. Biochem. Mol. Biol.* **49**, 361–399 [CrossRef Medline](#)
- Daffé, M., Crick, D. C., and Jackson, M. (2014) Genetics of capsular polysaccharides and cell envelope (glyco)lipids. *Microbiol. Spectr.* **2**, 14 [Medline](#)
- Angala, S. K., Palčková, Z., Belardinelli, J. M., and Jackson, M. (2018) Covalent modifications of polysaccharides in mycobacteria. *Nat. Chem. Biol.* **14**, 193–198 [CrossRef Medline](#)
- Škovierová, H., Larrouy-Maumus, G., Pham, H., Belanová, M., Barilone, N., Dasgupta, A., Mikusová, K., Gicquel, B., Gilleron, M., Brennan, P. J., Puzo, G., Nigou, J., and Jackson, M. (2010) Biosynthetic origin of the galactosamine substituent of arabinogalactan in *Mycobacterium tuberculosis*. *J. Biol. Chem.* **285**, 41348–41355 [CrossRef Medline](#)
- Wheat, W. H., Dhoub, R., Angala, S. K., Larrouy-Maumus, G., Dobos, K., Nigou, J., Spencer, J. S., and Jackson, M. (2015) The presence of a galactosamine substituent on the arabinogalactan of *Mycobacterium tuberculosis* abrogates full maturation of human peripheral blood monocyte-derived dendritic cells and increases secretion of IL-10. *Tuberculosis* **95**, 476–489 [CrossRef Medline](#)
- Bhamidi, S., Scherman, M. S., Rithner, C. D., Prenni, J. E., Chatterjee, D., Khoo, K.-H., and McNeil, M. R. (2008) The identification and location of succinyl residues and the characterization of the interior arabinan region allows for a model of the complete primary structure of *Mycobacterium tuberculosis* mycolyl arabinogalactan. *J. Biol. Chem.* **283**, 12992–13000 [CrossRef Medline](#)
- Delmas, C., Gilleron, M., Brando, T., Vercellone, A., Gheorghiu, M., Rivière, M., and Puzo, G. (1997) Comparative structural study of the mannosylated-lipoarabinomannans from *Mycobacterium bovis* BCG vaccine strains: characterization and localization of succinates. *Glycobiology* **7**, 811–817 [CrossRef Medline](#)
- Weber, P. L., and Gray, G. R. (1979) Structural and immunochemical characterization of the acidic arabinomannan of *Mycobacterium smegmatis*. *Carbohydr. Res.* **74**, 259–278 [CrossRef Medline](#)
- Hunter, S. W., Gaylord, H., and Brennan, P. J. (1986) Structure and antigenicity of the phosphorylated lipopolysaccharide antigens from the leprosy and tubercle bacilli. *J. Biol. Chem.* **261**, 12345–12351 [Medline](#)
- Torrelles, J. B., Khoo, K. H., Sieling, P. A., Modlin, R. L., Zhang, N., Marques, A. M., Treumann, A., Rithner, C. D., Brennan, P. J., and Chatterjee, D. (2004) Truncated structural variants of lipoarabinomannan in *Mycobacterium leprae* and an ethambutol-resistant strain of *Mycobacterium tuberculosis*. *J. Biol. Chem.* **279**, 41227–41239 [CrossRef Medline](#)
- Torrelles, J. B., Sieling, P. A., Arcos, J., Knaup, R., Bartling, C., Rajaram, M. V., Stenger, S., Modlin, R. L., and Schlesinger, L. S. (2011) Structural differences in lipomannans from pathogenic and nonpathogenic mycobacteria that impact CD1b-restricted T cell responses. *J. Biol. Chem.* **286**, 35438–35446 [CrossRef Medline](#)
- Torrelles, J. B., Sieling, P. A., Zhang, N., Keen, M. A., McNeil, M. R., Belisle, J. T., Modlin, R. L., Brennan, P. J., and Chatterjee, D. (2012) Isolation of a distinct *Mycobacterium tuberculosis* mannose-capped lipoarabinomannan isoform responsible for recognition by CD1b-restricted T cells. *Glycobiology* **22**, 1118–1127 [CrossRef Medline](#)
- Ortalo-Magné, A., Andersen, A. B., and Daffé, M. (1996) The outermost capsular arabinomannans and other mannoconjugates of virulent and avirulent tubercle bacilli. *Microbiology* **142**, 927–935 [CrossRef Medline](#)
- Guérardel, Y., Maes, E., Briken, V., Chirat, F., Leroy, Y., Loch, C., Strecker, G., and Kremer, L. (2003) Lipomannan and lipoarabinomannan from a clinical isolate of *Mycobacterium kansasii*: novel structural features and apoptosis-inducing properties. *J. Biol. Chem.* **278**, 36637–36651 [CrossRef Medline](#)
- Slauch, J. M., Lee, A. A., Mahan, M. J., and Mekalanos, J. J. (1996) Molecular characterization of the oafA locus responsible for acetylation of *Salmonella typhimurium* O-antigen: OafA is a member of a family of integral membrane trans-acylases. *J. Bacteriol.* **178**, 5904–5909 [CrossRef Medline](#)
- Thanweer, F., Tahiliani, V., Korres, H., and Verma, N. K. (2008) Topology and identification of critical residues of the O-acetyltransferase of serotype-converting bacteriophage, SF6, of *Shigella flexneri*. *Biochem. Biophys. Res. Commun.* **375**, 581–585 [CrossRef Medline](#)
- Thanweer, F., and Verma, N. K. (2012) Identification of critical residues of the serotype modifying O-acetyltransferase of *Shigella flexneri*. *BMC Biochem.* **13**, 13 [CrossRef Medline](#)
- Yamaryo-Butte, Y., Rainczuk, A. K., Lea-Smith, D. J., Brammananth, R., van der Peet, P. L., Meikle, P., Ralton, J. E., Rupasinghe, T. W., Williams, S. J., Coppel, R. L., Crellin, P. K., and McConville, M. J. (2015) Acetylation of trehalose mycolates is required for efficient MmpL-mediated membrane transport in Corynebacterineae. *ACS Chem. Biol.* **10**, 734–746 [CrossRef Medline](#)
- Belardinelli, J. M., Yazidi, A., Yang, L., Fabre, L., Li, W., Jacques, B., Angala, S. K., Rouiller, I., Zgurskaya, H. I., Sygusch, J., and Jackson, M. (2016) Structure-function profile of MmpL3, the essential mycolic acid transporter from *Mycobacterium tuberculosis*. *ACS Infect. Dis.* **2**, 702–713 [CrossRef Medline](#)
- Driessen, N. N., Stoop, E. J., Ummels, R., Gurcha, S. S., Mishra, A. K., Larrouy-Maumus, G., Nigou, J., Gilleron, M., Puzo, G., Maaskant, J. J., Sparrius, M., Besra, G. S., Bitter, W., Vandenbroucke-Grauls, C. M., and Appelmek, B. J. (2010) *Mycobacterium marinum* MMAR\_2380, a predicted transmembrane acyltransferase, is essential for the presence of the mannose cap on lipoarabinomannan. *Microbiology* **156**, 3492–3502 [CrossRef Medline](#)
- Yamazaki, Y., Danelishvili, L., Wu, M., Macnab, M., and Bermudez, L. E. (2006) *Mycobacterium avium* genes associated with the ability to form a biofilm. *Appl. Environ. Microbiol.* **72**, 819–825 [CrossRef Medline](#)
- Etienne, G., Villeneuve, C., Billman-Jacobe, H., Astarie-Dequeker, C., Dupont, M.-A., and Daffé, M. (2002) The impact of the absence of glycopeptidolipids on the ultrastructure, cell surface and cell wall properties, and phagocytosis of *Mycobacterium smegmatis*. *Microbiology* **148**, 3089–3100 [CrossRef Medline](#)
- Yamazaki, Y., Danelishvili, L., Wu, M., Hidaka, E., Katsuyama, T., Stang, B., Petrofsky, M., Bildfell, R., and Bermudez, L. E. (2006) The ability to form biofilm influences *Mycobacterium avium* invasion and translocation of bronchial epithelial cells. *Cell Microbiol.* **8**, 806–814 [CrossRef Medline](#)
- Rose, S. J., and Bermudez, L. E. (2014) *Mycobacterium avium* biofilm attenuates mononuclear phagocyte function by triggering hyperstimulation and apoptosis during early infection. *Infect. Immun.* **82**, 405–412 [CrossRef Medline](#)
- Pelacic, V., Jackson, M., Reyrat, J. M., Jacobs, W. R., Jr., Gicquel, B., and Guilhot, C. (1997) Efficient allelic exchange and transposon mutagenesis in *Mycobacterium tuberculosis*. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10955–10960 [CrossRef Medline](#)
- Grzegorzewicz, A. E., Pham, H., Gundi, V. A., Scherman, M. S., North, E. J., Hess, T., Jones, V., Gruppo, V., Born, S. E., Korduláková, J., Chavadi, S. S., Morisseau, C., Lenaerts, A. J., Lee, R. E., McNeil, M. R., and Jackson, M. (2012) Inhibition of mycolic acid transport across the *Mycobacterium tuberculosis* plasma membrane. *Nat. Chem. Biol.* **8**, 334–341 [CrossRef Medline](#)
- Morita, Y. S., Velasquez, R., Taig, E., Waller, R. F., Patterson, J. H., Tull, D., Williams, S. J., Billman-Jacobe, H., and McConville, M. J. (2005) Compart-

- mentalization of lipid biosynthesis in mycobacteria. *J. Biol. Chem.* **280**, 21645–21652 [CrossRef Medline](#)
37. Stadthagen, G., Sambou, T., Guerin, M., Barilone, N., Boudou, F., Kor-duláková, J., Charles, P., Alzari, P. M., Lemassu, A., Daffé, M., Puzo, G., Gicquel, B., Rivièrè, M., and Jackson, M. (2007) Genetic basis for the biosynthesis of methylglucose lipopolysaccharides in *Mycobacterium tuberculosis*. *J. Biol. Chem.* **282**, 27270–27276 [CrossRef Medline](#)
38. De, P., McNeil, M., Xia, M., Boot, C. M., Hesser, D. C., Deneff, K., Rithner, C., Sours, T., Dobos, K. M., Hoft, D., and Chatterjee, D. (2018) Structural determinants in a glucose-containing lipopolysaccharide from *Mycobacterium tuberculosis* critical for inducing a subset of protective T cells. *J. Biol. Chem.* **293**, 9706–9717 [CrossRef Medline](#)
39. Kaur, D., McNeil, M. R., Khoo, K. H., Chatterjee, D., Crick, D. C., Jackson, M., and Brennan, P. J. (2007) New insights into the biosynthesis of myco-bacterial lipomannan arising from deletion of a conserved gene. *J. Biol. Chem.* **282**, 27133–27140 [CrossRef Medline](#)
40. Berg, S., Starbuck, J., Torrelles, J. B., Vissa, V. D., Crick, D. C., Chatterjee, D., and Brennan, P. J. (2005) Roles of the conserved proline and glycosyl-transferase motifs of EmbC in biosynthesis of lipoarabinomannan. *J. Biol. Chem.* **280**, 5651–5663 [CrossRef Medline](#)
41. Martin, A., Camacho, M., Portaels, F., and Palomino, J.-C. (2003) Resazurin microtiter assay plate testing of *Mycobacterium tuberculosis* susceptibilities to second-line drugs: rapid, simple, and inexpensive method. *Antimicrob. Agents Chemother.* **47**, 3616–3619 [CrossRef Medline](#)
42. Eskandarian, H. A., Odermatt, P. D., Ven, J. X. Y., Hannebelle, M. T. M., Nievergelt, A. P., Dhar, N., McKinney, J. D., and Fantner, G. E. (2017) Division site selection linked to inherited cell surface wave troughs in mycobacteria. *Nat. Microbiol.* **2**, 17094 [CrossRef Medline](#)