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REVIEWS

Structural Remodeling Creates Diversity in Lipid Repertoire of *Mycobacterium tuberculosis*

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Abstract | *Mycobacterium tuberculosis* (Mtb) cell wall houses some of the most diverse lipids known. This enormous diversity is a product of interesting evolutionary redesigning of common folds. In this review, we present three examples of changes at increasing levels of complexity in common folds to generate diversity in biochemical and biological function. PKS18, a type-III polyketide synthase, exhibits specificity for longer acyl chains due to subtle psi angle changes in a canonical thiolase fold. A small insertion in a common acetyl-CoA synthetase fold redirects transfer of activated acyl chains to polyketide biosynthesis, whereas the counterparts transfer them to Coenzyme-A for degradation. Evolution of a large hydrophobic platform in the C-terminal domain of a short-chain dehydrogenase family member and adapting reduction as a release strategy enhances the diversity of lipids manifolds. These three examples underline a general theme of divergent evolution leading to plurality of functions with different levels of changes to the existing folds in the context of complex virulent lipid synthesis in Mtb.

1 Introduction

In the "omics" era, comparative analysis of sequences and structures has become a general norm in understanding not only the spatial and temporal biological functions but also the evolution of life. This is feasible because the genetic information contained in DNA, RNA and protein sequences is vast[1] and structural information adds a totally new dimension to that understanding. The technological advancements along with developments in computational methods have aided in this explosion of biological sequence information. This information can be consolidated and extended to biomolecules, whose functions have not been identified, by means of the most fundamental relationship called homology. Comparison of three-dimensional structures in conjunction with sequence information leads to a deeper understanding of evolutionary relationships between proteins, particularly of those sequences exhibiting identities in the twilight zone (20–30%).[2] The evolutionary relationships in proteins might be constrained to support a similar catalytic chemistry, called divergent evolution,[3,4] or convergent evolution,[5,6] where similar chemistry is reinvented in completely unrelated systems. These evolutionary processes act to seek an effective solution to a biochemical problem faced by an organism. The current review describes three examples of divergent evolution where these processes have resulted in generating enormous diversity in the lipid repertoire of *Mycobacterium tuberculosis* (Mtb).

2 *Mycobacterium tuberculosis*-Diversity of Lipids

Tuberculosis, an airborne infectious disease, is caused by rod-shaped bacillus Mtb. This epidemic has reached alarming proportions, accounting for ~9 million new cases and 1.4 million TB deaths in 2011.[7] Its deadly nexus with human immunodeficiency virus (HIV) along with poor drug compliance leading to multidrug-resistant tuberculosis (MDR-TB; resistant to at least, isoniazid and rifampicin) and extensively drug-resistant TB (XDR-TB; MDR-TB plus resistant to fluoroquinolone and amikacin, kanamycin or capreomycin) have worsened the situation.

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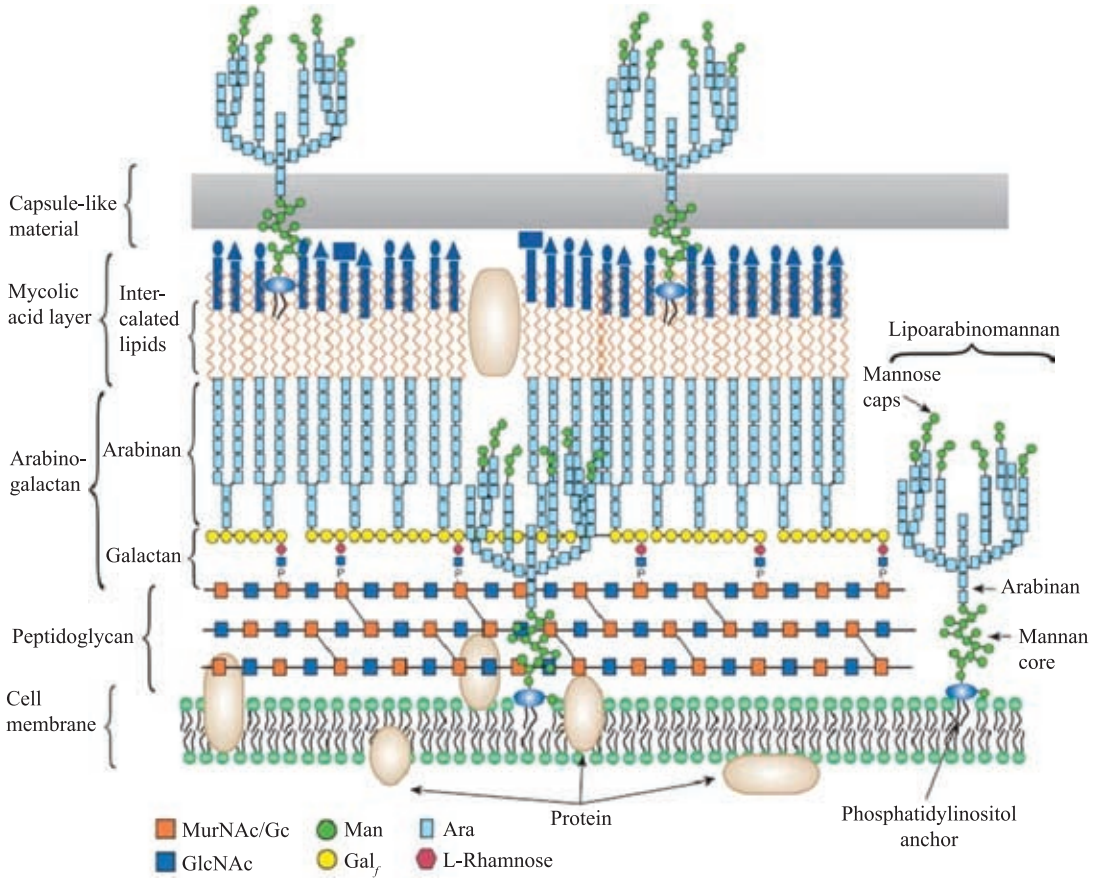


Figure 1: Schematic representation of *M. tuberculosis* cell envelope. [Adapted representation[8]].

A major factor making Mtb a successful pathogen is its unique cell envelope composition, consisting of a plethora of chemically and structurally diverse lipids (Fig 1) like lipomannan (LM), lipoarabinomannan (LAM), mycolyl arabinogalactan (MAG), trehalose dimycolate (TDM), sulfolipids (SL), polyacyl trehaloses (PAT), phthiocerol dimycocerosate (DIM) to name a few.[9,10] These lipids are instrumental in establishing the infection and tide over the hostile host immune responses.[11,12] The efforts over last two decades have shown that multiple paralogs of enzyme systems like Polyketide synthases (PKSs), fatty acid degradation enzymes (FadDs, FadEs, FadBs, FadAs etc) are at the heart of redesigning the lipid metabolic network in Mtb. These multiple paralogs present interesting cases of enzyme evolution which ultimately dictates the fate of the common lipid pool in Mtb.

3 Phi-Psi Switch and Novel Metabolites in Mtb

Polyketide synthases (PKSs) constitute a group of enzyme systems known for their role in the biosynthesis of an extensive range of secondary metabolites such as rapamycin, erythromycin, tetracycline, lovastatin, resveratrol, to name a few. There are three types of PKSs based on domain architecture and enzymology. Type-I and Type-II PKSs are giant modular multifunctional proteins that utilize the 4'-phosphopantetheine moiety on acyl carrier protein (ACP) to funnel acyl groups as substrates to the growing polyketide intermediates. Type-III PKSs are simpler and smaller enzyme systems that employ acyl-CoA thioesters as substrates to make polyketides. Despite the differences, all these enzymes utilize a common strategy of repetitive decarboxylative Claisen condensation of simple acyl-CoA thioesters to produce broad range of structurally and chemically diverse polyketide products.[13–15]

The sequencing of Mtb genome led to the discovery of PKSs,[16] which were later demonstrated as key players in the biosynthesis of virulent lipids of Mtb cell wall. The type-III polyketide synthases (PKSs), belonging to chalcone synthase (CHS) superfamily of enzymes have been extensively studied from plants.[17,18] Mycobacterial type-III PKSs such as PKS18 (MtbPKS18) and PKS11 display a remarkable specificity to accept long-chain

acyl-CoA thioesters (C12 to C20) as starter substrates for biosynthesis of linear polyketides, which eventually cyclize to produce long-chain tri- and tetraketide α-pyrones.[19,20]

The high resolution crystal structure of PKS18 (PDB ID: 1TED) is one of the first known among the bacterial type III PKSs (Fig 2a). The canonical features of thiolase fold enzymes like the overall architecture, the extensive dimeric interface, catalytic triad of *Cys-His-Asn* and the invariant phenylalanine (role in the decarboxylation reaction) have been found to be conserved.[21] Detailed structural analyses unveiled subtle differences in the vicinity of the substrate binding region of *Medicago sativa* CHS (msCHS; PDB ID: 1CGZ) and MtbPKS18. These changes were achieved by subtle manipulation of the ψ–torsion angles (Fig 2b; Table 1) resulting in the opening of a huge tunnel (~20 Å) in the molecule distal to the dimeric interface. Conserved inter-subunit interactions forming the base of the active-site cavity in CHS family of enzymes are missing in MtbPKS18. Thus, the tunnel in MtbPKS18 is unique in its location and disposition with respect to the catalytic site as compared to the related thiolase-fold enzymes. The 20 Å long novel tunnel was observed in PKS18 extending from the active site to the surface, which was hypothesized to be involved in acyl-chain accommodation of varying chain lengths.

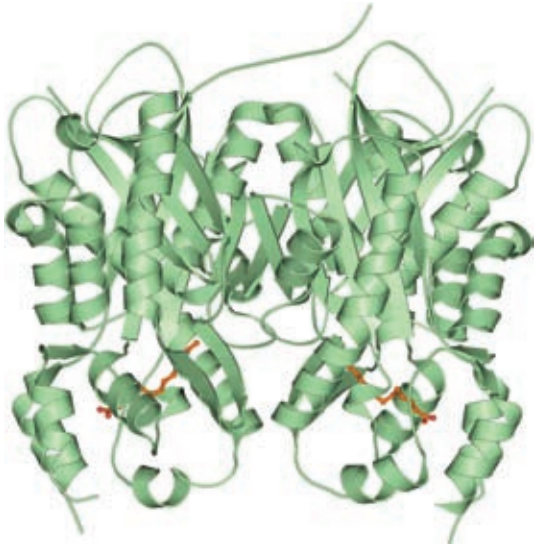


Figure 2a: The cartoon representation of the canonical thiolase fold in MtbPKS18.

The side chains of the amino acid residues T144, C205, and A209 seem to be crucial determinants of the tunnel length in PKS18. A C205F mutant displayed altered enzyme specificity where it could accept only short acyl chains due to the block in the tunnel created by the bulkier phenyl side chain. The side-chains lining cavity at the active-site and the tunnel confer the preferred geometry to facilitate a favorable cavity size and tunnel length for incorporation of longer-chain

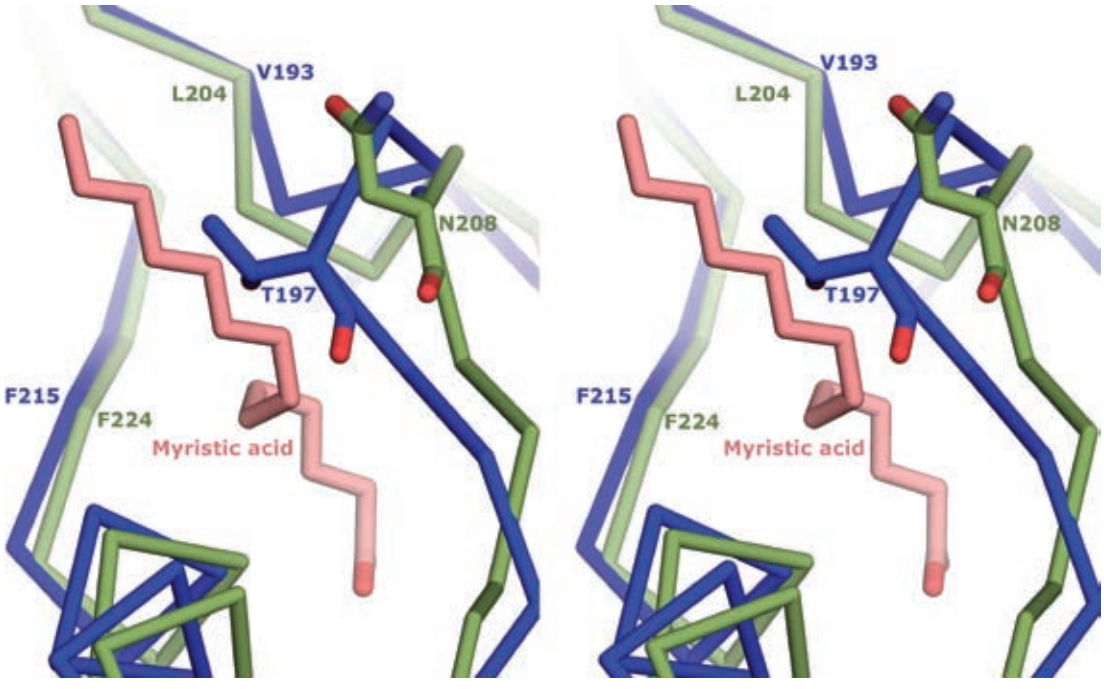


Figure 2b: Stereo diagram showing the subtle modulations in psi angle of residues lining the substrate-binding tunnel in MtbPKS18 (Green) as compared to MsCHS (Blue) for accommodation of long acyl chains.

Table 1: Comparison of dihedral angles of various thiolase fold members showing psi-angle similar to PKS18-like tunnel as compared to *Medicago sativa* CHS (MsCHS).

1CGZ Ms CHS	ϕ ψ	1TED Mtb PKS18	ϕ ψ	4JAR Mtb PKS11	ϕ ψ	3E1H Nc PKSIII	ϕ ψ	1U0M Sc THNS	ϕ ψ
V193	–136, **121**	L204	–121, **81**	L167	–112, **79**	V185	–138, **101**	F167	–120, **73**
T194	–76, **–4**	C205	–69, **–44**	C168	–60, **–40**	S186	–118, **–8**	C168	–59, **25**
A195	–54, –31	S206	–51, –29	S169	–45, –39	T187	–67, –27	S169	–65, 21
V196	–80, **–22**	V207	–70, **–16**	L170	–78, **–2**	T188	–66, **–8**	L170	–76, **–7**
T197	–102, **–17**	N208	–107, **14**	T171	–86, **4**	M189	–100, **16**	C171	–109, **21**

acyl-CoA substrates unlike the plant type-III PKSs. These cyclic ketides have not yet been detected in extracts from Mtb cultures. However, it is well established that mycobacterial PKSs generate linear polyketide products like mycocerosic acid, phthiocerol, mycoceranic acid, mycoketide, phenolphthiocerol, and mycolipenic acid. Mycobacterial PKSs employ thioesterase or acyl transferases to release these linear products; cyclization may be used as another alternate release pathway in some of the PKSs. Further investigation is required to completely decipher the biology surrounding these unusual metabolites in Mtb.

4 Novel Tunnel Making Key Metabolites in Other Organisms

The type-III PKS from the filamentous fungi *Neurospora crassa* (NcPKSIII) produces resorcinolic products by using long-chain starter substrates accommodated in the long substrate-binding tunnel, very similar to that of PKS18.[22] Structure-based mutational studies to block the tunnel not only altered the fatty acyl chain specificity *in vitro*, but also resulted in change of cyclization pattern affecting the product profile. The expression of NcPKSIII protein could be found during all three stages of the fungi-mycelia, conidia and ascospores. There is a possible functional importance of these resorcinolic lipids in *N. crassa* which is yet to be identified. Interestingly, in the case of *Azotobacter vinelandii* it has been suggested that the type-III PKS synthesizes resorcinolic lipids responsible for cyst formation using a similar tunnel as observed in PKS18. Thus, the evolution of this long substrate-binding tunnel in the common thiolase fold contributes towards the promiscuity in the starter substrates of type-III PKS, and hence the enormous diversity in the products they produce.[23]

5 Insertion in Acetyl-CoA Synthetase Fold and Fate of Mycobacterial Lipids

Free fatty acids are converted to acyl-CoA in a two-step process by a common acetyl-CoA synthetase (ANL) family of enzymes. The fatty acids are first activated as acyl-adenylates using adenosine triphosphate (ATP) molecule and then to acyl-CoA molecule through thioesterification to a Coenzyme-A molecule.[24–26] These acyl-CoA molecules then participate in β-oxidation pathways for energy generation and also in other cellular processes like signal transduction, protein acylation etc. The ANL superfamily (**A**cyl-CoA synthetases, **N**RPS adenylation domains and **L**uciferase enzymes) of enzymes encompass different proteins that perform similar chemical reaction employing different acyl chains like fatty acids, amino acids, coumarins, luciferins etc. Despite differences in the substrates, all these enzymes perform activation of acyl chains using ATP and then couple them with nucleophiles like thiol groups or molecular oxygen. The active site is at the interface of a large N-terminal domain, and a small C-terminal domain and residues from both these domains participate in the reaction. The most remarkable feature of the members of ANL superfamily is that they undergo large conformational change referred to as 'domain alternation'.[27] This remarkable conformational change results in reorganization of the residues at the interface allowing the two steps of the reaction to occur with the first feeding into the second. The precise molecular events resulting in these domain motions are yet to be uncovered and have been reviewed elsewhere.[28–30] The consensus emerging from these studies indicate that the hydrolysis of ATP and release of pyrophosphate (PPi) result

in dramatic change in electrostatic charge at the interface which triggers the domain rotation.

Sequence analysis of Mtb genome revealed that there are 36 paralogs of FadD, which catalyze the conversion of free fatty acids to acyl-CoA molecules. It was then demonstrated biochemically that 12 of these paralogs, called Fatty acyl-AMP ligases (FAALs), were able to catalyse the first adenylation step, while others, called Fatty acyl-CoA ligases (FACLs) were able to convert the fatty acids to acyl-CoA molecules.[31]

The three-dimensional structure determination of a representative FAAL, FAAL28, led to the identification of an insertion at a strategic location in Acetyl-CoA synthetase fold (Fig 3). The deletion of this insertion resulted in 'gain of function' in FAALs while incorporation of this insertion in FACLs resulted in 'loss of function'.[32] The insertion can thus be attributed to the inability of FAALs to react with CoA. Mechanistically, FAALs present an interesting naturally occurring variant to the domain rotation paradigm of ANL superfamily of enzymes. Evolution of insertion in FAALs is perplexing given the conservation of residues involved in CoA-binding. It has been observed that FAALs are neighbors to PKS genes in the genome, while FACLs are randomly distributed in the genome. The conservation of residues involved in CoA-binding in FAALs, presence of CoA derived pantetheine arm in acyl carrier protein (ACP) domain of PKS and proximity of FAAL and PKS prompted the hypothesis of FAALs acting as agents diverting activated fatty acids to PKS for biosynthesis. The biochemical demonstration of FAALs ability to transfer activated fatty acids to PKS led to a new paradigm in lipid metabolism of *Mycobacterium tuberculosis*. The evolution of an insertion in a common acetyl CoA-synthetase fold resulted in two enzyme systems, FAALs and FACLs, where FAALs divert the common lipid pool towards biosynthesis while FACLs divert it to other metabolic process like β-oxidation.

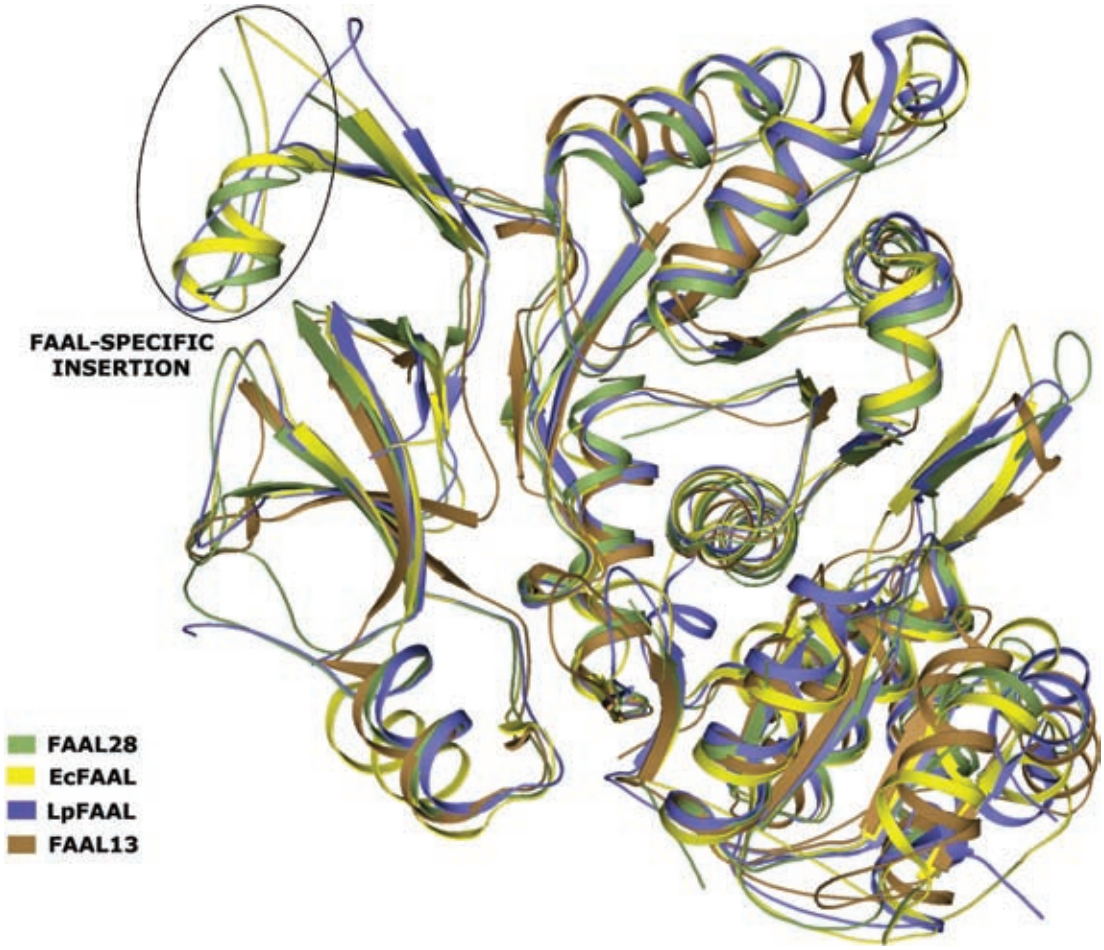


Figure 3: Structural alignment of FAALs showing FAAL-like insertion (Mtb: FAAL28-Green, *E.coli*: EcFAAL-Yellow and *L.pneumophila*: LpFAAL-Blue) as compared to FACLs (Mtb: FACL13-Brown).

6 The Rigidity of Insertion and FAALs in Other Organisms

The insertion of FAALs is present in the highly variable sub-domain-C of the N-terminal domain (Fig 4a). The insertion is anchored in the N-terminal domain by non-polar contacts and even a slight perturbation that destabilizes this anchorage leads to 'gain-of-function' in FAALs. The non-polar residues in N-terminal domain of FAALs are replaced by polar ones in other members of acetyl-CoA synthetase superfamily (Fig 4b). The presence of insertion in the sub-domain-C and

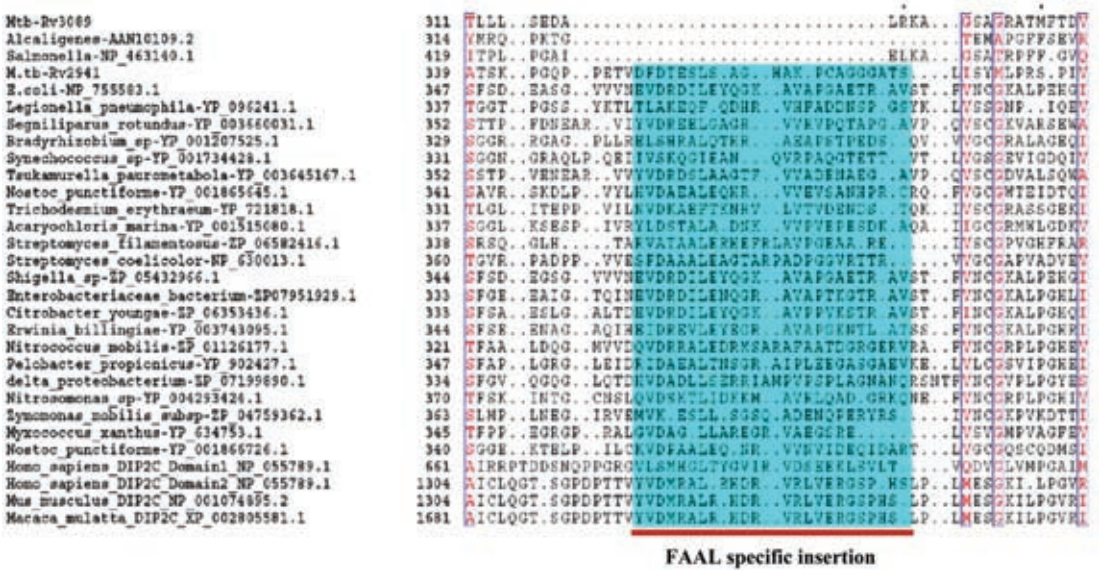


Figure 4a: Structure-based sequence alignment showing presence of FAAL-like insertion in many organisms.

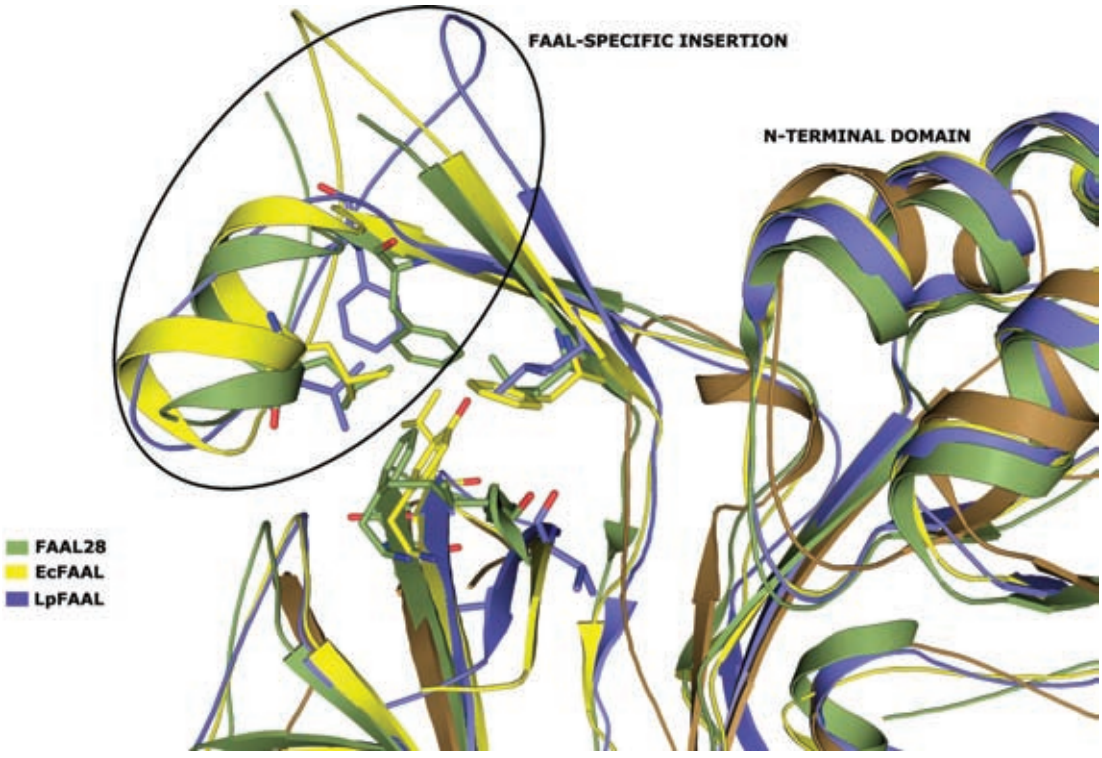


Figure 4b: The FAAL-specific insertion and its non-polar interaction network with N-terminal domain (through residues represented as sticks) in FAAL28 (Mtb; Green), EcFAAL (E.coli; Yellow) and LpFAAL (L.pneumophila; Blue).

its anchorage through non-polar contacts were used for *in silico* identification of FAALs in various organisms.[33]

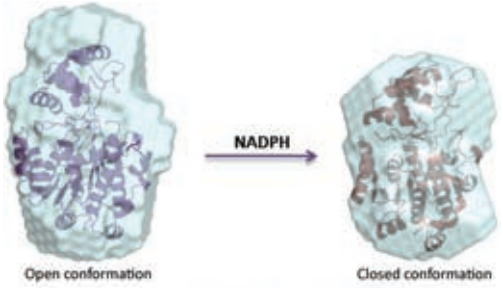
The FAAL-like proteins identified by this search were later characterized structurally and biochemically by independent groups.[34,35] The rigidity of insertion is the reason why FAALs fail to perform the thioesterification reaction. However, it is also intriguing how ACP would be able to overcome this rigid insertion enabling domain motions and the thioesterification reaction to happen. The most interesting finding of this *in silico* analysis was identification of FAAL-like proteins in higher eukaryotic organisms. For instance, DIP2, a conserved protein from *C.elegans* to *H.sapiens,* thought to be involved in development,[36,37] has the insertion and non-polar contacts for anchorage. It will be interesting to explore why insertion-based mechanism has been adapted in higher eukaryotes where PKSs and NRPSs are absent.

7 Conformational Changes and Cofactor Recycling for Reductive Release of Lipids

Multimodular megasynthetases, NRPSs and PKSs produce complex metabolites using a thiotemplate based assembly-line enzymology. These products are offloaded by thioesterase domain (TE) using hydrolysis or a lesser known Short-chain dehydrogenases/reductases (SDR) family member, the reductase domain (R), releasing the 4'-Phosphopantetheine arm tethered substrate by reduction as an aldehyde or an alcohol.[38,39] Subsequently, specific modifications of this hydroxyl moiety allow myriad variations in the products otherwise not possible with hydrolysis carried out by a TE domain. In mycobacterial species, NRPSs are known to be involved in myxochelin and glycopeptidolipid biosynthesis. Genome mining in *Mycobacterium* species led to the identification of several multifunctional proteins that possess R domains at their C-terminus which may follow similar nature of catalysis for offloading the products.

The crystal structure of R_{NRP} (R-domain of *nrp;* Rv0101) from Mtb (Fig 5a) revealed interesting facets of evolutionary patchwork in the canonical architecture of SDR family, apart from providing insights into the mechanistic basis of R-domain mediated catalysis in context of NRPSs.[40] The R_{NRP} shows the canonical features of SDR family such as the two-domain architecture, the catalytic triad of *Thr/Ser-Tyr-Lys* and the well conserved Rossmann fold. Interestingly, the NAD(P)H-binding pocket was occupied by a loop between β5 of the Rossmann fold and the following α–helix. Biochemical assays demonstrated that the R-domain would perform nonprocessive $[2 + 2]e^-$ reduction forming both aldehyde and alcohol. This process essentially would involve extrusion of the intermediate aldehyde allowing cofactor recycling facilitated by conformational changes. These conformational changes were established in both Mtb and

Figure 5a: Structure of R_{NRP} showing residues of the hydrophobic region in C-terminal domain (Yellow).



Protein	R_g (Å)	D_{max} (Å)
R_{NRP} (open)	24.4±0.4	75
R_{NRP}+NADPH (closed)	22.6±0.4	66
R_{GPL} (open)	26.8±0.9	89
R_{GPL}+NADPH (closed)	23.4±0.4	70

Figure 5b: *Ab initio* envelopes of open and closed conformation of R_{NRP} obtained from SAXS studies along with a table showing corresponding comparative changes in Radius of gyration (R_g) and Maximum dimension (D_{max}).

Mycobacterium smegmatis (M.smeg) using extensive small-angle x-ray scattering studies that show significant changes in the radius of gyration (R_g) and maximum particle size (D_{max}) during the binding process (Fig 5b).

8 Unique Hydrophobic Pocket and Expanding Substrate Repertoire of SDR Family

An interesting feature with the R-domain is the presence of a large C-terminal substrate-binding domain with a unique hydrophobic pocket, atypical of SDR family of enzymes. The evolution of such a hydrophobic pocket in substrate-binding domain allows the capture of long acyl chain usually found in the complex lipidic metabolites made by NRPS and PKS of mycobacterial species. The remarkable structural similarity of the central β-sheet architecture of Rossmann-fold with the α/β hydrolase fold of the TE domain provides an insight into the replacement of TE domain with R-domain as the offloading domain during the course of evolution. The release of acyl chains by reduction also allows additional modifications such as glycosylations as in the case of glycopeptidolipids (GPL) in M.smeg and hence enhancing the diversity of metabolites made by these assembly-line multifunctional biosynthetic enzymes like NRPS and PKS. The functional analysis of R-domain from M.smeg indicates that the formation of a terminal alcohol is crucial for the subsequent glycosylation in GPL biosynthesis.[41,42] Although it very clear that R_{NRP} can reduce acyl chains to alcohols, the implications of the formation of such a lipophilic peptide containing a terminal alcoholic group in Mtb biology needs to be addressed.

9 Discussion

Mtb is a storehouse of a plethora of lipids. These structurally and chemically diverse lipids are products of interplay between fatty acid biosynthesis machinery and polyketide synthases. Interesting themes of evolution have been unraveled on the path towards understanding the metabolism of these unique lipids in Mtb. These evolutionary events have resulted in a remarkable diversity in cell lipids, and such themes were also found to be adapted in other organisms for different functions. The first theme was subtle changes in dihedral angles of residues lining the susbtrate-binding tunnel resulting in remarkable substrate specificity of a type-III PKS, PKS18. This novel tunnel allows accommodation of a wide range of fatty acids increasing the repertoire of metabolites, a common thiolase fold could generate. The second theme was a small insertion mutation in a common acetyl-CoA synthetase fold which shunts the common lipid pool towards biosynthesis. Fatty-acyl AMP ligases transfer the activated fatty acids to pantetheine arm of acyl carrier protein unlike other members of the superfamily that transfer the acyl-adenylates to pantetheine of CoA. These activated fatty acids shunted towards biosynthesis are used to generate the enormously diverse lipids of Mtb. This specificity results from the insertion which hampers the domain motions which is then relieved only by ACP. However, the precise role of insertion-based mechanism in effecting domain motions is still not understood. In the third theme, evolution of an R-domain in multi-modular biosynthetic enzymes likes NRPS and PKS allowed reductive release of complex metabolites. This was also accompanied by evolution of a novel hydrophobic pocket in SDR family of enzymes resulting in broad substrate tolerance and accommodation of longer chains usually present in mycobacterial lipids. In addition to broad substrate tolerance, the free hydroxyl group produced by reductive release allows a platform for glycosylation tremendously increasing the diversity in the lipids being produced. In conclusion, these three examples present three different evolutionary themes resulting in remarkable diversity in biochemical functions using minimal changes to the existing folds, which in case of Mtb resulted in diversity of lipids of its cell wall.

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References

1. Higgs PG & Attwood TK (2005) *Bioinformatics and molecular evolution* (Blackwell, Malden, MA; Oxford) pp xiii, 365 p., 312 p. of plates.
2. Doolittle RF (1986) *Of urfs and orfs: a primer on how to analyze derived amino acid sequences* (University Science Books, Mill Valley, CA) pp vii, 103 p.
3. Gerlt JA & Babbitt PC (2001) Divergent evolution of enzymatic function: mechanistically diverse superfamilies and functionally distinct suprafamilies. (Translated from eng) *Annual review of biochemistry* 70:209–246.
4. Todd AE, Orengo CA, & Thornton JM (1999) Evolution of protein function, from a structural perspective. *Current opinion in chemical biology* 3(5):548–556.
5. Almonacid DE, Yera ER, Mitchell JB, & Babbitt PC (2010) Quantitative comparison of catalytic mechanisms and

overall reactions in convergently evolved enzymes: implications for classification of enzyme function. *PLoS computational biology* 6(3):e1000700.
6. O'Boyle NM, Holliday GL, Almonacid DE, & Mitchell JB (2007) Using reaction mechanism to measure enzyme similarity. *Journal of molecular biology* 368(5):1484–1499.
7. WHO WHO (2012) Global Tuberculosis Control: WHO Report 2011. *Australian and New Zealand Journal of Public Health* 36(5):497–498.
8. Brennan PJ & Crick DC (2007) The cell-wall core of Mycobacterium tuberculosis in the context of drug discovery. *Current topics in medicinal chemistry* 7(5):475–488.
9. Anderson RJ (1941) Structural Peculiarities of Acid-fast Bacterial Lipids. *Chem Rev* 29(2):225–243.
10. Goren MB (1972) Mycobacterial lipids: selected topics. *Bacteriological reviews* 36(1):33–64.
11. Briken V, Porcelli SA, Besra GS, & Kremer L (2004) Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response. *Molecular microbiology* 53(2):391–403.
12. Hunter RL, Olsen MR, Jagannath C, & Actor JK (2006) Multiple roles of cord factor in the pathogenesis of primary, secondary, and cavitary tuberculosis, including a revised description of the pathology of secondary disease. *Annals of clinical and laboratory science* 36(4):371–386.
13. Hopwood DA (1997) Genetic Contributions to Understanding Polyketide Synthases. *Chem Rev* 97(7):2465–2498.
14. Katz L & Donadio S (1993) Polyketide synthesis: prospects for hybrid antibiotics. *Annual review of microbiology* 47:875–912.
15. Khosla C, Gokhale RS, Jacobsen JR, & Cane DE (1999) Tolerance and specificity of polyketide synthases. *Annual review of biochemistry* 68:219–253.
16. Cole ST, *et al.* (1998) Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. *Nature* 393(6685):537–544.
17. Austin MB & Noel JP (2003) The chalcone synthase superfamily of type III polyketide synthases. *Natural product reports* 20(1):79–110.
18. Schrödei J (2000) Chapter Three: The family of chalcone synthase-related proteins: Functional diversity and evolution. *Recent Advances in Phytochemistry*, eds John T. Romeo RILV & Vincenzo De L (Elsevier), Vol Volume 34, pp 55–89.
19. Gokulan K, *et al.* (2013) Crystal structure of Mycobacterium tuberculosis polyketide synthase 11 (PKS11) reveals intermediates in the synthesis of methyl-branched alkylpyrones. *The Journal of biological chemistry* 288(23):16484–16494.
20. Saxena P, Yadav G, Mohanty D, & Gokhale RS (2003) A new family of type III polyketide synthases in Mycobacterium tuberculosis. *The Journal of biological chemistry* 278(45):44780–44790.
21. Sankaranarayanan R, *et al.* (2004) A novel tunnel in mycobacterial type III polyketide synthase reveals the structural basis for generating diverse metabolites. *Nature structural & molecular biology* 11(9):894–900.
22. Goyal A, *et al.* (2008) Structural insights into biosynthesis of resorcinolic lipids by a type III polyketide synthase in Neurospora crassa. *Journal of structural biology* 162(3):411–421.
23. Sankaranarayanan R (2006) A type III PKS makes the DIFference. *Nature chemical biology* 2(9):451–452.
24. Lindmark DG (1980) Energy metabolism of the anaerobic protozoon Giardia lamblia. *Molecular and Biochemical Parasitology* 1(1):1–12.
25. Muller M (1988) Energy metabolism of protozoa without mitochondria. *Annual review of microbiology* 42:465–488.
26. Reeves RE, Warren LG, Susskind B, & Lo HS (1977) An energy-conserving pyruvate-to-acetate pathway in Entamoeba histolytica. Pyruvate synthase and a new acetate thiokinase. *The Journal of biological chemistry* 252(2):726–731.
27. Gulick AM (2009) Conformational dynamics in the Acyl-CoA synthetases, adenylation domains of non-ribosomal peptide synthetases, and firefly luciferase. *ACS chemical biology* 4(10):811–827.
28. Hisanaga Y, *et al.* (2004) Structural basis of the substrate-specific two-step catalysis of long chain fatty acyl-CoA synthetase dimer. *The Journal of biological chemistry* 279(30):31717–31726.
29. Kochan G, Pilka ES, von Delft F, Oppermann U, & Yue WW (2009) Structural snapshots for the conformation-dependent catalysis by human medium-chain acyl-coenzyme A synthetase ACSM2 A. *Journal of molecular biology* 388(5):997–1008.
30. Yonus H, *et al.* (2008) Crystal structure of DltA. Implications for the reaction mechanism of non-ribosomal peptide synthetase adenylation domains. *The Journal of biological chemistry* 283(47):32484–32491.
31. Trivedi OA, *et al.* (2004) Enzymic activation and transfer of fatty acids as acyl-adenylates in mycobacteria. *Nature* 428(6981):441–445.
32. Arora P, *et al.* (2009) Mechanistic and functional insights into fatty acid activation in Mycobacterium tuberculosis. *Nature chemical biology* 5(3):166–173.
33. Goyal A, Verma P, Anandhakrishnan M, Gokhale RS, & Sankaranarayanan R (2012) Molecular basis of the functional divergence of fatty acyl-AMP ligase biosynthetic enzymes of Mycobacterium tuberculosis. *Journal of molecular biology* 416(2):221–238.
34. Hayashi T, Kitamura Y, Funa N, Ohnishi Y, & Horinouchi S (2011) Fatty acyl-AMP ligase involvement in the production of alkylresorcylic acid by a Myxococcus xanthus type III polyketide synthase. *Chembiochem: a European journal of chemical biology* 12(14):2166–2176.
35. Zhang Z, *et al.* (2011) Structural and functional studies of fatty acyl adenylate ligases from E. coli and L. pneumophila. *Journal of molecular biology* 406(2):313–324.
36. Mukhopadhyay M, *et al.* (2002) Cloning, genomic organization and expression pattern of a novel Drosophila gene, the disco-interacting protein 2 (dip2), and its murine homolog. *Gene* 293(1–2):59–65.

37. Tanaka M, *et al.* (2010) DIP2 disco-interacting protein 2 homolog A (Drosophila) is a candidate receptor for follistatin-related protein/follistatin-like 1–analysis of their binding with TGF-beta superfamily proteins. *The FEBS journal* 277(20):4278–4289.
38. Gokhale RS, Hunziker D, Cane DE, & Khosla C (1999) Mechanism and specificity of the terminal thioesterase domain from the erythromycin polyketide synthase. *Chemistry & biology* 6(2):117–125.
39. Du L & Lou L (2010) PKS and NRPS release mechanisms. *Natural product reports* 27(2):255–278.
40. Chhabra A, *et al.* (2012) Nonprocessive [2 + 2]e-off-loading reductase domains from mycobacterial nonribosomal peptide synthetases. *Proceedings of the National Academy of Sciences of the United States of America* 109(15):5681–5686.
41. Krzywinska E & Schorey JS (2003) Characterization of genetic differences between Mycobacterium avium subsp. avium strains of diverse virulence with a focus on the glycopeptidolipid biosynthesis cluster. *Veterinary microbiology* 91(2–3):249–264.
42. Vats A, *et al.* (2012) Retrobiosynthetic approach delineates the biosynthetic pathway and the structure of the acyl chain of mycobacterial glycopeptidolipids. *The Journal of biological chemistry* 287(36):30677–30687.

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