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DNA metabolism in *Mycobacterium leprae*

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Introduction

Understanding the molecular basis underlying the hallmark features of *Mycobacterium leprae*, such as its parasitism and extraordinarily slow growth rate, has stimulated research into the biology of this pathogen for decades, but it is through the completion of its genome sequence that a quantum leap of progress has been achieved.[1] In this review, we analyse the genes in *M. leprae* that are involved in the synthesis and salvage of purines and pyrimidines and in DNA replication and repair in an attempt to uncover the relationship between the massive gene decay observed in the *M. leprae* genome and its DNA metabolic capacity. This analysis has provided insights into possible mechanisms for the genomic deterioration in the leprosy bacillus and supplements the sparse biochemical data hard won from this organism.

Nucleotide biosynthesis and salvage

Purine and pyrimidine compounds are central to nucleic acid synthesis and byproducts of the purine pathway are essential components of energy metabolism and protein synthesis. Cellular levels of purines also affect induction of the stringent response and are important for the regulation of many other genes.

DE NOVO SYNTHESIS OF PURINES

Purine biosynthesis proceeds in a stepwise fashion that is conserved across prokaryotes and eukaryotes. A complete pathway for the production of purines has been preserved in *M. leprae* but it has a minimal gene complement for the conversion of ribose-5-phosphate to inosine monophosphate (IMP); it does not possess *purT* or *purU* (encoding the formate-dependent phosphoribosylglycinamide formyltransferase II and formyltetrahydrofolate

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deformylase, respectively) which restricts the addition of the C-8 position to 5′-phosphoribosyl-1-glycinamide to the action of phosphoribosylglycinamide formyltransferase (*purN*). In contrast to *E. coli*, where phosphoribosylformylglycinamide synthase activity comprises PurL alone,[2] mycobacteria follow the *B. subtilis* model[3] with the *M. leprae* activity comprising PurL, PurQ and PurS (ML2219A).

For a genome so peppered with pseudogenes, it is surprising that *M. leprae* has retained three *guaB* genes (*guaB1-B3*) encoding IMP dehydrogenase, which converts IMP to xanthine monophosphate (XMP). By analogy with *Mycobacterium tuberculosis*,[4] GuaB2 probably provides most of the IMP dehydrogenase activity in *M. leprae*. A candidate for the *M. tuberculosis purR* regulator[5,6] is Rv3575c,[7] the counterpart of which is a pseudogene in *M. leprae* (ML0338), suggesting that regulation of this pathway may differ between mycobacterial species. The presence of a complete pathway for *de novo* purine biosynthesis deduced from the genome sequence is not consistent with the conclusion that *M. leprae* is dependent upon the host as a source for the purine ring.[8,9] However, regulatory impairment and low levels of constitutive gene expression might explain the observed purine auxotrophy of *M. leprae*. Levels of phosphoribosylamine glycine ligase (*purD*) and phosphoribosylaminoimidazole carboxylase ATPase (*purK*) in *M. leprae* were undetectable compared to adenylosuccinate lyase (*purB*).[10]

DE NOVO SYNTHESIS OF PYRIMIDINES

M. leprae also possesses a complete gene set for *de novo* pyrimidine synthesis, in accordance with experimental observations.[11,12] Although the biosynthetic components of the pathway are conserved with *M. tuberculosis*, the *M. leprae pyrR*, which would be responsible for UMP-dependent attenuation of the pyrimidine operon, is a pseudogene. The context of the biosynthetic genes is largely conserved between these two species, but the genes encoding the first three steps in the conversion of glutamine to dihydroorotate differ in their operonic structure, suggesting that this biosynthetic cluster may be differentially regulated.

BIOSYNTHESIS OF DEOXYRIBONUCLEOTIDES FROM RIBONUCLEOTIDES

The reduction of ribonucleotides to deoxyribonucleotides is catalysed by ribonucleotide reductases (RNRs), which are classified on the basis of their O_2 dependence. The O_2 dependent, class I family of RNRs is divided into subclasses I(a) and I(b), represented by NrdAB and NrdEF respectively, which utilize NDPs as substrates. Class II RNRs (NrdJ) are indifferent to O_2, catalyse the reduction of NTPs and utilize adenosylcobalamin as a co-factor, and the anaerobic class III (NrdDG) enzymes generate a glycyl radical from S-adenosylmethionine and an iron-sulfur cluster to reduce NTPs. The RNR gene repertoire of *M. leprae* is simpler than that of *M. tuberculosis*,[4] consisting only of *nrdE* and *nrdF*. As in *M. tuberculosis*, *nrdE* is operonic with *nrdH* and *nrdI*, which encode the electron transport component of NrdEF and a protein with stimulatory properties for ribonucleotide reduction, respectively.[13] dADP, dGDP and dCDP are derived directly by reduction of the corresponding NDPs and all other enzymes involved in the production of dNTPs are conserved (nucleotide diphosphate kinase, dUTPase and thymidylate synthase).

SALVAGE PATHWAYS

Salvage pathways control the intracellular levels of nucleosides and bases produced from catabolism of nucleic acids and nucleotides and those taken up from the extracellular milieu.

Analysis of the genome sequence suggests that the purine salvage pathway of *M. leprae* is largely intact, whereas the pyrimidine pathway is fragmented, consistent with experimental evidence suggesting that salvage of purines plays a more important role than that of pyrimidines.[14] The uptake and utilization of adenosine and hypoxanthine has been demonstrated in macrophage-maintained leprosy bacilli,[15] and dephosphorylation of nucleotides and subsequent importation of nucleosides has also been shown to occur in *M. leprae*.[8,14]

The purine salvage pathway converting adenosine to AMP via adenine by *pnp* or *deoD* (purine nucleoside phosphorylases) and *apt* (adenine phosphoribosyltransferase) is not complete in *M. leprae*; although the *pnp-apt* pathway is probably functional in *M. tuberculosis, pnp* and *apt* are pseudogenes in *M. leprae*. ML0707 is annotated as *deoD*, a guanosine/inosine/adenosine phosphorylase of the PNP family I of phosphorylases, but domain analysis indicates that it belongs instead to the PNP family II and would therefore catalyse the degradation of all purine nucleosides except adenosine and deoxyadenosine. However, conversion of adenosine to adenine is a minor pathway in *E. coli* and the major pathway, which cycles adenosine to IMP is conserved in *M. leprae* (*add*, ML0707 and *hpt*). Guanosine can be cycled to guanosine monophosphate (GMP) in a similar fashion (ML0707, *hpt*). In *Escherichia coli* and *Bacillus subtilis*, xanthine and guanine are only cycled to IMP via GMP. Neither xanthine phosphoribosyltransferase (*xpt/gpt*), which recycles xanthine to XMP, nor GMP reductase (*guaC*) was identified in the *M. leprae* genome, but since IMP dehydrogenase and GMP reductase are closely related enzymes with similar tertiary structures, GuaB1 and/or GuaB2 may function as GMP reductase. Finally, although high levels of adenosine kinase activity were detected in *M. leprae*,[10] we could not correlate a coding region(s) with this activity, suggesting that a non-orthologous enzyme may be present.

M. leprae has been shown to incorporate exogenous pyrimidines as bases or nucleosides, with uracil alone being able to supply all of its pyrimidine requirements.[14] Incorporation of ^{3}H-thymidine has also been reported in *M. leprae* isolates.[15] However, we could not identify genes for thymidine kinase (*tdk*), thymidine phosphorylase (*deoA*) or uridine/cytidine kinase (*udk*). Moreover, the genes encoding uracil phosphoribosyltransferase (*upp*, *pyrR*) are non-functional, which would preclude the direct conversion of uracil to UMP. *M. leprae* has also acquired a eukaryotic-like uridine phosphorylase (ML2177) that is not found in *M. tuberculosis*, for the interconversion of uracil and uridine (and possibly also thymine and thymidine, and uracil and deoxyuridine). Cytidine may also be converted to uridine by cytidine deaminase (*cdd*) and deoxycytidine to deoxyuridine by deoxyctidine deaminase (ML2507). In the absence of an enzyme to produce UMP from uracil or uridine, it is surprising that an intact *cdd* gene still remains, as this would shuttle cytidine to a metabolic dead-end. These observations, in conjunction with experimental evidence,[14,15] argue in favour of the presence of non-orthologous uridine and/or thymidine kinases.

DNA replication

The highlights of our comparative analysis of the DNA replication and repair gene complements of *M. leprae* and *M. tuberculosis* are summarized below.

INITIATION/ELONGATION/PROCESSING PROTEINS

Proteins involved in initiation (DnaA), accessory proteins (mIHF, HU, RNA-P), those in the pre-priming complex (SSB, DnaB), DnaG primase and DNA gyrase are all conserved

in *M. leprae*, but DnaC is absent, suggesting that the replicative complex does not require DnaC to load DnaB. Like *M. tuberculosis*, *M. leprae* lacks RNase HI, suggesting that TopA and RNase HII provide initiation specificity. The RNA primers for replication could be processed by the 5′–3′ exonuclease activity of PolI, but as previously noted, the RLEP elements flanking the *polA* gene may affect its expression and hence compromise its processing function.[16]

REPLICATIVE COMPLEX

Although most information on the bacterial replication complex (DNA PolIII holoenzyme) has been gained from studies in *E. coli*,[17] recent work has elucidated the subunit composition of a Gram-positive PolIII[18] against which that of *M. leprae* can be compared. *M. leprae* PolIII comprises τ/γ (*dnaZX*), δ' (*holB*) and δ (ML0603) subunits. As in *Streptomyces coelicolor*, the polymerase activity of *M. leprae* PolIII is provided by the Gram-negative type α subunit encoded by *dnaE1*.[19] This structure differs from that of low G+C Gram positive organisms, which contain a PolC subunit.[18] Mycobacteria also possess a second, Gram-negative type α subunit gene (*dnaE2*), but in *M. leprae*, this is a pseudogene. Studies in *E. coli* have revealed that in the core holoenzyme, α is tightly associated with ϵ, the *dnaQ*-encoded proofreading subunit, which serves to markedly increase the fidelity of replication.[20,21] In addition to its 3′–5′ exonuclease activity, ϵ is required for maximal DNA synthesis[22] and also plays an important structural role within the core.[23] We therefore consider the fact that *M. leprae dnaQ* is a pseudogene to be highly significant.[4] Apart from less efficient (slower) polymerase action, the absence of a proofreading subunit of the replicative polymerase has major implications for the maintenance of genome integrity particularly as *M. leprae* also lacks the genes involved in *mutLS*-based mismatch repair.[4] The absence of two of the three pillars of replication fidelity in *M. leprae* places the full burden of fidelity on the α subunit, which led us to speculate that enhanced base selectivity by α might be the only mechanism available to avoid error catastrophe[21,24] in this organism.[4]

The loss of *dnaQ* may be reflected in the astonishing number of base substitutions and frameshifts in the *M. leprae* genome. *M. leprae* isolates also display variable numbers of TTC repeats that are not found in *M. tuberculosis* or *Mycobacterium avium*.[25] Since expansion and contraction of triplet repeats map to mutations in *dnaQ*,[26] this form of genomic diversification argues against the existence of an alternate proofreading subunit in *M. leprae*. This conclusion is also consistent with the absence of intact 3' exonuclease-encoding genes that could serve this function. Finally, as replication in *M. leprae* may be inherently inefficient and error-prone, it is not surprising that this organism has also dispensed with its UmuC-like, error-prone polymerases.

DNA repair

As in other metabolic processes, considerable decay is evident in the repertoire of DNA repair genes in *M. leprae*. However, as highlighted below, the decay has mainly served to reduce the number of genes encoding redundant or overlapping biochemical functions while leaving the major repair pathways intact.

DAMAGE REVERSAL

Although *M. tuberculosis* is well equipped to reverse bases damaged by O-alkylation and pyrimidine dimers, *M. leprae* has retained only the suicidal DNA methyltransferase, Ogt, which is required for mutation avoidance.

MISMATCH REPAIR

All mycobacterial genomes sequenced to date lack the highly conserved, *mutLS*-based mismatch repair pathway,[4,27] implying that as in *M. tuberculosis*, base substitution and simple frameshift mutations may become fixed in *M. leprae* due to the lack of strand discrimination inherent in repair by enzymes such as MutY and UvrABC.[27]

EXCISION REPAIR

M. leprae possesses at least one copy of each of the genes required for the base excision repair (BER) of lesions created by hydrolytic, alkylative and oxidative and damage, namely uracil DNA glycosylases (*ung* and possibly ML1105), 3-methylpurine DNA glycosylases (*tagA*, ML1351), glycosylase/lyases (*fpg*, *nth*) and an apyrimidinic/apurinic endonuclease (*nfo*), but its restricted gene repertoire reflects the functional redundancy of BER activities. As such, *M. leprae* possesses neither *nei* nor *xthA*, but as these genes play minor roles in the presence of functional *fpg*, *nth* and *nfo*,[28,29] their absence is tolerable. Both *M. tuberculosis* and *M. leprae* possess AlkB (ML0190), which acts on alkylated single-stranded (ss) DNA at replication forks.[30] Since AlkB is not widely distributed amongst the genomes sequenced to date, its presence suggests that mycobacteria may be particularly prone to alkylation damage.[31]

M. leprae also possesses a complete gene complement for nucleotide excision repair (NER) of bulky lesions and adducts that cannot be repaired by BER. The NER process is initiated by UvrABC, which recognizes and binds to the lesions, with UvrD and PolI being responsible for further processing.

RECOMBINATIONAL REPAIR

The recombinational proficiency of *M. leprae* is evident in the structure of its genome, which shows that multiple homologous recombination events have occurred between repetitive sequences.[1] It is therefore not surprising that *M. leprae* contains a complete set of genes for homologous recombination. As in other mycobacteria, the *recA* gene, which plays a pivotal role in the recombinational repair of strand breaks, is probably *lexA*-regulated and damage-inducible in *M. leprae*.[32–34] The function of *M. tuberculosis* RecA is well described[35] and *M. leprae* RecA is assumed to have a similar activity. In *E. coli*, a number of pathways exist for the initial processing of double-stranded DNA breaks to ss substrates for recombination, each featuring the action of exonucleases and helicases. In *E. coli*, 3′ invasive ends for recombination can be generated by RecBCD or by the exonuclease/helicase pairs, RecE/RecT or RecQ/RecJ. Mycobacteria do not contain *recE*, *recT*, *recO* or *recJ*, and are unique amongst the Gram-positive organisms sequenced thus far in that they possess a Gram-negative type RecBCD, rather than the analogous AddAB found in low G + C Gram-positives.[36] *M. leprae* possesses neither of these systems, but it does possess homologues of

an archaeal exonuclease (ML1155) and helicase (ML1312) belonging to the *recB* family of exonucleases/helicases,[37] in addition to several other helicases (*helY*, ML2157 and ML1624) and exonucleases (*sbcD* (ML1119), *xseAB*) which could provide the break-processing function.

In *E. coli,* only one pathway has been shown to process the resulting ss DNA substrate further, and this RecFOR pathway is conserved in *M. leprae*. Helicase II (UvrD) is proposed to play an important part in RecBCD$^-$ cells in the RecF pathway of recombination, and in this context it is notable that *M. leprae* has retained both *uvrD* and *uvrD2* genes. Strand exchange is subsequently promoted by the actions of TopA and DNA Pol I, both of which are present in *M. leprae,* and as in other organisms, RecA, together with RuvAB and RecG would carry out branch migration and RuvC and DNA ligase would finally resolve the heteroduplex.

Conclusions

While acknowledging the limitations inherent in deducing gene function from 'sequence gazing', our analysis has nonetheless provided some intriguing insights into the genetics of DNA metabolism in *M. leprae*, which could guide future studies. High on our list of priorities would be to investigate the functional relationship between defects in components of replication fidelity and the process of gene decay.

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