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REVIEWS

Spotlight

Mannogen-ing Central Carbon Metabolism by *Leishmania*

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Leishmania parasites synthesize mannogens, a unique type of storage carbohydrate, from finely tuned interactions between synthesis and degradation by a family of mannosyltransferase/phosphorylases (MTPs) newly discovered by Sernee *et al*. The crucial roles of mannogen in regulating central carbon metabolism and *in vivo* virulence suggest the potential of MTPs as promising drug targets.

Typically, eukaryotes synthesize and store glucose polymers, such as starch and glycogen, as their main carbohydrate reserves, the synthesis and degradation of which play key roles in regulating energy production and central carbon metabolism. *Leishmania* and closely related trypanosomatids lack such canonical carbohydrate reserves. Instead, they synthesize mannogens, a family of linear oligosaccharides made up of β-1,2-linked mannose residues (2–60 in length, depending on development stage) [1]. Mannogen is highly abundant during the pathogenic stages of *Leishmania mexicana,* and mutants deficient in GDP-mannose biosynthesis lack mannogen (as well as other mannose-containing surface virulence factors) and are completely avirulent in animal models [1]. Mannogen is rapidly catabolized when parasites are exposed to glucose-limiting conditions, suggesting that it can serve as an energy/carbon reserve, like glucose-based polymers, and contribute to parasite survival in the mammalian host.

Previously, the McConville group showed that mannogen synthesis involves a mannose phosphate primer and subsequent extension by GDP-Man-dependent mannosyltransferases [2]. Now, Sernee *et al.* have identified a tandem gene array in *Leishmania* encoding seven enzymes (termed MTP1–7) with homology to bacterial mannosidases/phosphorylases [3]. Biochemical analysis, discussed below, established that these enzymes have MTP activities, and a null mutant lacking all enzymes (*Δmtp1-7*) showed a complete absence of mannogen, thereby establishing a key role for this gene cluster.

A Family of Glycosyltransferase-phosphorylases Mediate Mannogen Synthesis and Degradation

Biochemical assays using purified recombinant enzymes and synthetic mannogen analogs demonstrated that MTP1 and 2 had GDP-Man-dependent β-1,2-mannosyltransferase activity (for mannogen elongation) and the reverse, GDP-dependent phosphorolytic activity (for mannogen degradation) (Figure 1). MTP3, 4, 6, and 7 showed strong phosphorolytic activity in the presence of orthophosphate (Pi), catalyzing mannogen degradation as well as the reverse phosphorylase activity towards mannogen elongation in the presence of mannose-1-phosphate (Man-1P). In addition to Man-1P, MTP3, 4, 6, and 7 could also use GDP-Man as the donor for mannogen elongation, indicating their dual mannosyltransferase activities (Figure 1). MTP5 was catalytically inactive *in vitro*, despite the presence of expected catalytic motifs.

The 3D crystal structures of *L. mexicana* MTP1, 2, and 4 revealed a five-bladed β-propeller fold surrounding the active site that shares similar architecture to those of bacterial GH130 proteins [4]. Structural differences in the catalytic pockets of MTP1/2 and MTP4 likely contribute to the different donor/acceptor specificities of these enzymes (Figure 1), and mutagenesis studies identified key amino acid residues that may distinguish the mannosyltransferase activity from phosphorylase activity.

To examine the functions of MTPs *in vivo*, *L. mexicana* mutants lacking either all or a subset of the seven *MTP* genes were generated and then complemented with various MTPs (singly or in combination). Based on qualitative and quantitative analyses of mannogen in transgenic parasites, MTP1 and MTP2 act cooperatively to prime the synthesis of long (>12) and short (2–10) mannogen oligomers, respectively. In contrast, MTP3, 4, 6, and 7 primarily act as phosphorylases, and exhibit only limited GDP-Man-transferase activity *in vivo* (Figure 1). Thus, MTPs have nonredundant functions in the cycling of mannogen *in vivo*.

Importantly, MTP activities are required for *L. mexicana* pathogenesis *in vivo*, as *Δmtp1–7* and *Δmtp3–7* mutants were unable to induce lesions or proliferate in highly susceptible BALB/c mice. Complementation with one or two *MTP*s partially restored virulence, suggesting that mannogen synthesis and turnover must be meticulously regulated for parasites to survive in the mammalian host. *Δmtp* mutant promastigotes were also hypersensitive to elevated temperature (33°C), low pH (5.5), and high or low glucose conditions. These results highlight the importance of mannogen cycling under varying nutrient conditions: when the glucose level is low, mannogen can be metabolized into monosaccharides to produce energy and a carbon source, while under nutrient-replete conditions, mannogen synthesis can reduce cytoplasmic glucose levels and protect parasites from the accumulation of

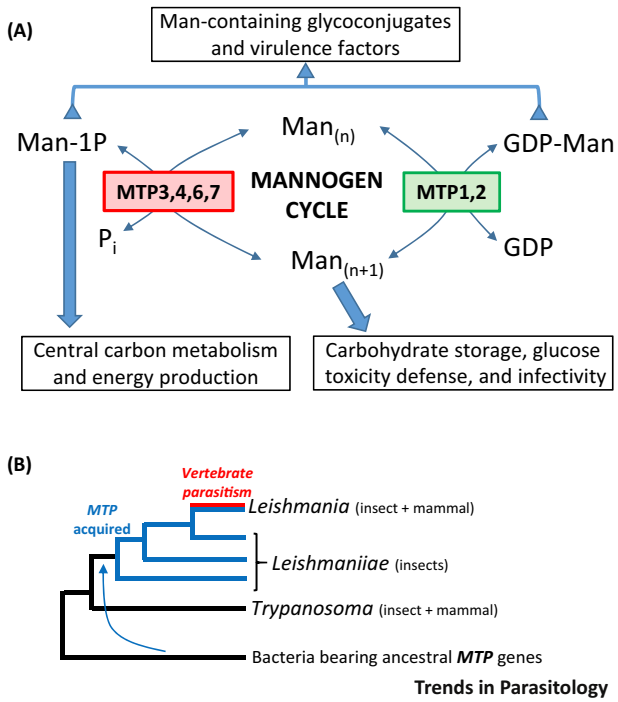

Figure 1. The Mannogen Cycle of *Leishmania*.
(A) The inferred roles of the dual-activity mannosyltransferase/phosphorylases (MTPs) towards mannogen synthesis (*MTP 1,2*) or degradation (*MTP 3,4,6,7*), and the key roles of mannogen and its precursor/products are shown. (B) Evolutionary origins of mannogen/MTP pathway. The ancestral *MTP* genes were likely acquired from bacteria, early in the evolution of the Leishmaniiae (which primarily reside within invertebrates, although *Phytomonas* also infect plants) and following the divergence of this group from the lineage leading to trypanosomes, which lack *MTP*s and mannogen (black lines). The presence of mannogen/MTPs (blue lines) predated, and may have facilitated, the transition of *Leishmania* to vertebrate parasitism (red lines). Adapted from Sernee *et al*. [3].

reactive oxygen species or other harmful metabolites [5] (Figure 1).

Beyond their role in energy homeostasis, mannogens potentially serve as a reservoir for the synthesis of mannose-rich glycoconjugates playing key roles in the parasite's infectious cycle, including lipophosphoglycan (LPG) and related phosphoglycans, as well as the abundant glycosylinositolphospholipids [6]. Their syntheses require mannose, GDP-Man and/or Man-1P, all of which arise from mannogen degradation. It is also possible that separate pools exist for mannogen synthesis/degradation from those needed for virulence glycoconjugates, as seen in the segregation of precursor pools for GPI anchor synthesis amongst various *Leishmania* glycoconjugate classes [7]. It will thus be interesting to see to what extent disruptions in mannogen synthesis carry over into the synthesis of virulence glycoconjugates, and their contribution to the pathogenesis defects seen in macrophage and mouse studies.

Evolutionary Origin of MTPs by Horizontal Transfer Preceded Transition to Vertebrate Parasitism by *Leishmania*

MTP orthologs are found exclusively in bacteria and certain trypanosomatid lineages, including *Leishmania*, *Phytomonas*, *Leptomonas*, and *Crithidia*. Phylogenetic analysis suggested that the MTP gene family was acquired by horizontal gene transfer after the separation of the subfamily Leishmaniinae from Trypanosomatidae (which lack mannogen). Acquisition of the mannogen-cycling pathway appears to have occurred well before the evolution of mammalian infectivity, as the earliest MTP-bearing parasites reside only within the invertebrate digestive track. This raises the possibility that, as in the mammalian stages, mannogens may play a role within the insect vector, where parasites must similarly buffer varying levels of nutrients following feeding and digestion. Potentially, acquisition of MTPs and the mannogen pathway may have contributed by 'pre-adapting' *Leishmania* for the transition to vertebrate parasitism (Figure 1).

Overall, this seminal study by Sernee *et al*. suggests that the dynamics of mannogen cycling play a pivotal role in regulating central carbon metabolism during the mammalian stage [3]. Multiple MTPs must work collaboratively *in vivo* to maintain a dynamic equilibrium between monosaccharides and mannogen, which may serve as a 'metabolic rheostat' to buffer changes in sugar uptake, energy consumption, and metabolites. Collectively, the uniqueness of mannogen in *Leishmania*, and its crucial roles in regulating central carbon metabolism *in vivo*, suggest that MTPs may represent promising drug targets, for which biochemical screens are already available [8].

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Forum

Multiple Blood Feeding: A Force Multiplier for Transmission

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Anopheles mosquitoes employ complex behavioral and physiological strategies to adapt to their environment. Here we show how altering the number of bites a mosquito takes per gonotrophic cycle (gonotrophic discordance) could raise the transmission potential of a mosquito population far above model predictions.

Violating a Common Assumption

Malaria transmission models rely on entomological parameters extrapolated from field-collected data [1]. Considering the complexity of mosquito behavior [2] and the paucity of data from the field, it is crucial to evaluate how model predictions rely on unknown quantities or uncertain assumptions. The malaria reproduction rate, R_0, represents the chance that a malaria parasite will find a competent vector, mature, and infect another human. So long as R_0 >1, transmission occurs, with new cases emerging faster than existing cases resolve. Drop below $R_0 = 1$, and net transmission ceases. One of the classic entomological parameters [3] used to compute R_0 is the human feeding rate (a), calculated as the product of the proportion of mosquito bites taken on a human (Q) and the number of total bites taken per gonotrophic cycle (f). However, f is overwhelmingly taken to equal 1, implying gonotrophic concordance (i.e., that the number of blood meals taken by a mosquito is assumed to be equivalent to the number of gonotrophic cycles) [2]. Numerous field studies indicate a violation of this assumption [2,4–6]. Gonotrophic discordance, the phenomenon of taking multiple blood meals per gonotrophic cycle, is also known as multiple blood feeding (MBF). This is distinct from interrupted feeding, where defensive behavior from the host causes temporary disengagement followed by a resumption of feeding [2]. Multiple blood feeding appears to be beneficial for *Anopheles* females, increasing fecundity, longevity, and resistance to insecticides [2,7], all of which could contribute to increased disease transmission. These factors notwithstanding, MBF directly increases the number of potentially infective bites, the impact of which we highlight here.

To assess the impact of MBF on model predictions, we show the effect of raising the number of bites per gonotrophic cycle on R_0, the entomological inoculation rate (EIR), the vectorial capacity (V), and the age-specific proportion and total number of living, infectious vectors in a population (I_i and N_I, respectively) (see Table S1 in the supplemental information online). We consider the effects of MBF in low-, moderate-, or high-transmission environments by altering values for other parameters that determine overall transmission intensity.

The Impact of Multiple Blood Feeding on Modeled Malaria Transmission

The equations evaluated here incorporate components of the Ross–MacDonald model [8]. The original purpose of these equations was to address a major shortcoming of Ross's theory: a lack of field-based entomological measurements [8]. Increasing the EIR, vectorial capacity, or N_I would be expected to increase R_0.

The EIR represents the number of infective bites an individual human receives per unit time. Adding additional bites per cycle (by increasing the feeding rate a) raises the EIR at a given transmission intensity (Figure 1A). Underestimating the number of bites per gonotrophic cycle unrealistically lowers the EIR, an effect intensified by increased transmission intensity.

The vectorial capacity (V), or daily reproduction rate of the disease, is the expected number of infective mosquito bites that would eventually arise from all the mosquitoes that bite a single