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Nat Rev Microbiol. 2016 December ; 14(12): 744–759. doi:10.1038/nrmicro.2016.141.***Treponema pallidum*, the syphilis spirochete: making a living as a stealth pathogen****Justin D. Radolf¹, Ranjit K. Deka², Arvind Anand³, David Šmajs⁴, Michael V. Norgard⁵, and X. Frank Yang⁶**¹Departments of Medicine, Pediatrics, Genetics and Genomic Science, Molecular Biology and Biophysics, and Immunology, UConn Health, Farmington, CT, USA²Department of Microbiology, The University of Texas Southwestern Medical Center, Dallas, TX, USA³Department of Medicine, UConn Health, Farmington, CT, USA⁴Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic⁵Department of Microbiology, The University of Texas Southwestern Medical Center, Dallas, TX, USA⁶Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN**Abstract**

The last two decades have seen a worldwide resurgence in infections caused by *Treponema pallidum* subsp. *pallidum*, the syphilis spirochete. The syphilis spirochete's well-recognized capacity for early dissemination and immune evasion has earned it the designation 'the stealth pathogen'. Despite the many hurdles to studying syphilis pathogenesis, most notably the inability to culture and to genetically manipulate *T. pallidum*, in recent years, considerable progress has been made in elucidating the structural, physiologic, and regulatory facets of stealth pathogenicity. In this Review, we integrate this eclectic body of information to garner fresh insights into the highly successful parasitic lifestyles of the syphilis spirochete and related pathogenic treponemes.

Pathogenic treponemes cause venereal syphilis, yaws, endemic syphilis, and pinta—multi-stage, infections that, although similar, can be differentiated based on clinical, epidemiologic, and geographic criteria^{1,2}. Only venereal syphilis (see Box 1 for a detailed description) is transmitted by sexual activity. The pathogenic treponemes are uncultivable, slow-growing microorganisms with identical flat-wave morphologies. They poorly tolerate desiccation, elevated temperature, and ambient oxygen tension, traits that explain why efficient transmission requires close personal contact^{1,2}. Landmark DNA-DNA hybridization studies^{3,4} showed that DNA from a venereal syphilis spirochete (Nichols strain) shared less than 5% homology with DNA from cultivatable treponemes but was indistinguishable from

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Conflicts of interest

The authors report no conflicts of interest.

DNA of the yaws spirochete (Gauthier strain). This work led to the reclassification of the agents of venereal syphilis, endemic syphilis, and yaws as *Treponema pallidum* subspecies *pallidum*, *endemicum*, and *pertenue*, respectively. Genomic sequencing has established that these subspecies are clonal but form distinct genetic clusters with venereal syphilis strains, the most recently evolved group, likely diverging from the *pertenue* cluster several thousand years ago⁵. Genomic sequencing also has revealed that *T. paraluiscuniculi* (reclassified as *Treponema paraluisleporidarum* ecovar Cuniculus⁶), the cause of venereal spirochetosis in rabbits, shares a common ancestor with human pathogenic treponemes⁷. WHO estimates that ~90 million people in Africa, Asia and the Western Pacific currently are at risk for yaws⁸. Globally, ~11 million people acquired syphilis in 2008⁹, with mother-to-child transmission occurring in nearly 2 million pregnancies¹⁰. *T. pallidum* is considered the most virulent subspecies because it routinely traverses blood-brain and maternal-fetal placental barriers¹¹.

Box 1

Venereal syphilis in brief

After an incubation period ranging from 9 to 90 days, during which spirochetes are already blood-borne, disease commences with the appearance of the hallmark ulcerative lesion, the chancre (A (<http://phil.cdc.gov>) and B (courtesy of Kevin Dieckhaus¹¹)), at the site of inoculation. Mucocutaneous lesions of secondary syphilis (C (with permission from Reference 27) and D (courtesy of Adriana Cruz and Juan Salazar)) appear 4 to 10 weeks later. This stage is associated with the highest incidence of spirochetemia, the greatest treponemal burdens in blood and tissues, and the highest titers in serodiagnostic tests¹¹. A pregnant woman with untreated secondary syphilis is at great risk of transmitting the disease to her fetus (E (<http://phil.cdc.gov>))²⁸. Invasion of the central nervous system in as many as 40% of individuals with untreated early syphilis sets the stage for potentially devastating neurologic complications years later¹¹. After weeks to months, the patient enters a period of latency during which, in the absence of a clinical relapse, the diagnosis can be made only by serologic testing. Latency is divided into early latent (infectious relapses and/or spirochetemia common) and late latent (relapses and/or spirochetemia unlikely) stages. Approximately 25% of patients will experience one or more secondary-like relapses during early latency, 90% of which occur in the first year. Approximately 30% of untreated patients will develop one of the recrudescence syndromes ('benign' gummas (F (<http://phil.cdc.gov>)), cardiovascular syphilis (G, aortic arch aneurysm), and neurosyphilis (H, right cerebellar infarct indicated by arrow, (<http://phil.cdc.gov>)) collectively designated tertiary syphilis¹¹.



Venereal syphilis is acquired when treponemes are inoculated onto the mucosa or skin during sexual contact. Spirochetes directly penetrate mucous membranes or they enter through breaches in skin produced by sexual activity¹². Attachment to host cells and the extracellular matrix is considered to be the crucial initial step of infection^{13,14}. Once below the epithelium, the spirochetes multiply locally and disseminate through the lymphatics and the bloodstream¹². *T. pallidum*'s flexuous, flat-wave morphology (Fig. 1A and B)¹⁵ enables it to penetrate tissues and vascular barriers throughout the body, while its periplasmic motility apparatus propels it forward via front-to-back undulations coordinated in response to poorly understood chemotactic signals^{16,17}. Although it is not clear how *T. pallidum* benefits by invading deep visceral and musculoskeletal tissues, reaching and surviving in distal skin and mucosal sites enhances opportunities for subsequent transmission.

Venereal syphilis can be thought of as a contest between the ability of *T. pallidum*, generally considered to be an extracellular pathogen, to avoid recognition and the adeptness of the host's innate and adaptive immune responses to 'track down' the pathogen¹². The spirochete's fragile outer membrane lacks lipopolysaccharide (LPS), the highly proinflammatory glycolipid found in Gram-negative bacteria¹⁸. Although *T. pallidum* expresses numerous lipoproteins capable of activating macrophages and dendritic cells (DCs) through Toll-like receptor (TLR) 2-dependent signaling pathways¹⁹, they are predominantly below the surface¹³. The paucity of surface-exposed pathogen-associated molecular patterns (PAMPs) enables the bacterium to undergo repeated bouts of dissemination that are poorly detected by innate immunity and also explains the lack of systemic inflammatory symptoms characteristic of the disease¹¹. The appearance of opsonic antibodies, which promote internalization, killing, and degradation of spirochetes within phagolysosomes, represents a turning point in the battle between host and pathogen^{19–21}. However, by liberating lipopeptides for binding to TLRs lining the interior of the phagosome and antigenic peptides for presentation to resident and locally recruited T cells, bacterial clearance and killing becomes a double-edged sword. The ensuing inflammatory response causes tissue damage that gives rise to clinical manifestations¹¹. Spirochetes replicating in tissues elicit a complex and variable inflammatory cell infiltrate consisting of macrophages, CD4⁺ and CD8⁺ T lymphocytes, and plasma cells, accompanied by varying degrees of endothelial cell swelling and proliferation. Production of IFN- γ by locally activated CD4⁺ and CD8⁺ T cells enhances the capacity of macrophages to internalize and degrade spirochetes but also bolsters their output of potentially tissue damaging, proinflammatory cytokines^{22–26}.

Importantly, spirochetes are not easily cleared by opsonic antibodies; in fact, during secondary and early latent syphilis, viable bacteria circulate despite high titers of anti-treponemal antibodies^{27,28}. How the pathogen burden is eventually decreased and latency established and maintained is a mystery. Passive immunization with sera from previously infected rabbits immune to rechallenge with *T. pallidum* suggests that antibodies play a critical role in suppressing spirochete burdens during latency^{29,30}, although a contribution by cellular immunity cannot be ruled out^{19,31}. Equally mysterious is where spirochetes reside during latency. Experiments in rabbits have suggested that *T. pallidum* may use hair follicles and nerves as protected niches³². *T. pallidum*'s capacity to survive for prolonged periods while hiding 'in plain sight' of humoral defenses represents its defining feature as a stealth

pathogen³³. In the remainder of this Review, we integrate an eclectic and sometimes puzzling body of information to garner fresh insights into a fundamental question of syphilis pathogenesis: how does *T. pallidum* make a living in its obligate human host?

Experimental roadblocks and new directions

T. pallidum was among the first major bacterial pathogens of humans to be identified, yet our knowledge of syphilis pathogenesis lags well behind that of other common bacterial infections³⁴. One of the main reasons for this is the syphilis spirochete's refractoriness to cultivation *in vitro*, despite intensive efforts dating almost from its discovery in 1906³⁵. In the 1940s, investigators defined incubation conditions and medium components that maintained treponemal motility and virulence for 6–8 days, although without replication³⁶. In the early 1980s, reproducible increases (20- to 100-fold) in replication, with full preservation of motility and virulence, were obtained by cultivating treponemes under microaerophilic conditions in the presence of cottontail rabbit epithelial (Sf1Ep) cells^{37,38}. Subsequent efforts to improve on these results by meticulously defining growth conditions and parameters yielded new information about *T. pallidum* growth requirements but only modest increases in replication without achieving continuous culture^{35,39}. Although the genomic sequence confirmed that *T. pallidum* depends upon its host for virtually all of its nutritional requirements¹⁸, it did not yield obvious, untried solutions to the cultivation problem³⁵. A contemporary bioinformatics-based analysis of the possible functions of the more than 400 'hypothetical' proteins encoded in the *T. pallidum* genome⁴⁰ may inform future attempts. Recent recognition of the importance of riboflavin uptake and flavin utilization for spirochete metabolism and energy generation may be pertinent as well^{41,42}. Therefore, investigators today still rely upon intratesticular inoculation of rabbits to isolate *T. pallidum* strains from clinical samples and propagate them for experimentation⁴³.

The once popular notion that *T. pallidum*'s poor surface antigenicity can be attributed to a pseudo-capsule of serum proteins and mucopolysaccharides⁴⁴ gave way in the early 1990s to overwhelming evidence that the bulk of its immunogenic molecules are subsurface and that its delicate outer membrane forms the protective barrier^{13,45}. Despite the formidable roadblock imposed by the cultivation barrier, compounded by the lack of a facile, inbred animal model for assessing protective immunity⁴⁶, our understanding of this unorthodox bacterial outer membrane and its interactions with host defenses has increased substantially in recent years^{13,47}. In parallel, there has been growing interest in the physiologic and regulatory foundations of stealth pathogenicity. Residence in a rich, relatively homeostatic environment has enabled *T. pallidum* to dispense with genes for *de novo* synthesis of nucleotides, fatty acids, vitamins, co-factors, amino acids, the tricarboxylic acid cycle, and oxidative phosphorylation¹⁸, resulting in the smallest of the spirochete genomes (~1.1 MB) and one of the smallest among pathogenic bacteria⁴⁸. During the course of genomic reduction, the bacterium has undergone remarkable adaptations that enable it to acquire all of its required nutrients and optimize their usage within diverse niches, while coping with exogenous and endogenous stress.

The face of stealth-the outer membrane

Studying the properties and composition of the outer membrane of *T. pallidum* has been, and remains, arduous^{13,45}. The quest for rare outer membrane proteins (OMPs) began in earnest with the discovery by freeze-fracture electron microscopy (Fig. 1C), recently confirmed by scanning probe microscopy (Fig. 1D), that *T. pallidum* contains ~100-fold fewer OMPs than *Escherichia coli*^{49,50}. How then does *T. pallidum* meet its nutritional requirements and carry out its complex parasitic lifestyle with a minimalist outer membrane? A partial answer may lie with the bacterium's slow (~30 h) rate of replication⁵¹, presumably an evolutionary 'compromise' between the density of OMPs needed for viability and the demands of stealth.

Tpr proteins

Little progress identifying rare OMPs was made until the genomic sequence revealed the existence of the *T. pallidum* repeat (Tpr) proteins, a 12 member paralogous family with sequence homology to the major outer sheath protein (MOSP) of *Treponema denticola*^{18,52,53}, which is a known pore-forming protein and adhesin⁵⁴. Of these, TprK (TP0897)^{21,52,55} has received the greatest attention because of its proposed role in immune evasion (see below), although its status as a *bona fide* OMP has been challenged^{56,57}. Except for BamA (TP0326, originally known as TP92⁵⁸), the *T. pallidum* genome does not encode proteins with a high degree of sequence homology to well-characterized OMPs of Gram-negative bacteria¹⁸. As an alternative to genome mining for OMP orthologs, we used a computational matrix to identify proteins predicted to adopt the hallmark conformation of an OMP, the β -barrel⁵⁷. The two highest ranked Tprs, TprC/D (TprC (TP0117) and TprD (TP0131) are identical in the Nichols strain) and TprI (TP0620), possess all of the properties expected of a rare OMP^{59,60}. The native proteins are low in abundance (~200 copies per cell), surface-exposed, and amphiphilic, whereas the folded recombinants form β -sheet-rich, heat-modifiable trimers that readily insert into artificial membranes. Similar to MOSP⁶¹, integration of TprC/D and TprI into liposomes results in permeability increases comparable to those produced by the archetypal porin, *E. coli* OmpF^{59,60}. Formation of large, non-selective channels could explain how these rare proteins function cooperatively to meet the spirochete's nutritional needs. With classical porins, the entire polypeptide forms the β -barrel⁶²; a pivotal discovery is that TprC/D and TprI are bipartite (Fig. 1E)^{59,60}. A C-terminal domain forms the β -barrel, while the N-terminal portion extends into the periplasm, anchoring the barrel to the murein sacculus. Consistent with this bipartite model, TprF, a truncated TprC/D/I ortholog of these Tprs lacking the C-terminal β -barrel domain, is periplasmic. Of note, these analyses provide a functional and topological template for the extended Tpr family^{61,63}.

BamA and outer membrane biogenesis

Among the non-Tpr proteins predicted to form β -barrels⁵⁷, BamA is of paramount importance. BamA is the essential central component of the β -barrel assembly machine (Bam) that catalyzes the insertion of newly exported OMPs into the outer membrane^{64,65}. Like other members of the Omp85 superfamily, BamA has a dual domain architecture consisting of an outer membrane-inserted, C-terminal β -barrel and periplasmic polypeptide transport-associated (POTRA) repeats (in this case, five, Fig. 1E)^{64,65}. As in other bacteria,

BamA in *T. pallidum* is part of a complex (~400 kDa) whose subunits, unidentified thus far, assist in the insertion process⁶⁴. Conceivably, the periplasmic chaperones Skp (TP0327) and SurA (TP1016), which ferry newly exported OMPs to the Bam apparatus⁶⁶, function together with the POTRA repeats as a gatekeeper that regulates the protein composition and content of the outer membrane. A homology model based upon the solved structure of *Neisseria gonorrhoeae* BamA⁶⁷ predicted that *T. pallidum* BamA contains a 16-stranded β -barrel in which short, weakly hydrogen-bonded β 1 and β 16 strands separate to allow lateral insertion of nascent OMPs into the outer membrane⁶⁵. The BamA barrel has eight extracellular loops, with three (L4, L6 and L7) forming a dome that occludes the barrel's extracellular opening, directing nascent OMPs laterally into the bilayer. Although much of the dome is poorly immunogenic, L4 contains a surface-exposed, immunodominant, opsonic epitope, a potential Achilles' heel likely imposed by functional constraints on the β -barrel.

Adhesins

In vitro studies well predating the genomics era demonstrated that *T. pallidum* can bind to a variety of mammalian cell types^{68,69} as well as extracellular matrix proteins, particularly laminin and fibronectin^{68,70}. Using bioinformatics to identify potential outer membrane proteins in combination with binding assays, potential adhesins for fibronectin (TP0155 and TP0483)⁷¹ and lamin (TP0751) were identified^{72,73}. In addition to being an adhesin, TP0751 is a zinc-dependent metalloprotease (hence, was named "pallilysin") that forms a complex with TP0751 (which contains a Von Willebrand factor type A domain) capable of degrading clots and extracellular matrix, which could facilitate both dissemination and attachment^{74,75}. The lipoprotein TP0136 is a fibronectin-binding adhesin with sequence heterogeneity among *T. pallidum* strains (Table 1)^{76,77}. Most recently, elegant gain of function experiments using *B. burgdorferi* as a surrogate genetic host have shown that the lipoprotein TP0435 (Tpp17) functions as a cytoadhesin⁷⁸.

Implications for limiting surface antigenicity

While there is consensus that resistance to antibody binding is the basis for immune evasion by *T. pallidum*^{12,55}, much remains to be learned about how the pathogen accomplishes this impressive feat and, conversely, the counter-measures used by the host. Early immunolabeling experiments suggested that the outer membrane of *T. pallidum* is antigenically inert^{79,80}. However, this simplistic notion is contradicted by biological assays showing that syphilitic infection does, in fact, induce antibodies that react with the bacterial surface¹⁹. Moreover, immunolabeling and opsonophagocytosis assays have revealed that *T. pallidum* populations are heterogeneous, consisting of antibody-binding and non-binding subpopulations, and that organisms that bind antibodies do so with markedly slow kinetics^{26,57,81}. These observations likely explain the longstanding paradox that clearance and persistence of *T. pallidum* occur simultaneously²⁶. At a given site, antibody binders would be slowly cleared, also 'feeding' inflammation, whereas immunoevasive non-binders would replicate locally and disseminate systemically.

How, then, does heterogeneity in surface antigenicity occur? With BamA, TprC/D, and TprI, the large majority of antibodies are directed against periplasmic domains^{60,64,65} and, are, therefore, ineffective for clearance. For those antibodies that are potentially opsonic, the

extremely low copy numbers of the target proteins likely limits their accessibility, as evidenced by the sizable percentage of treponemes that fail to become surface-labeled or opsonized by high-titer recombinant antisera against TprC/D or BamA^{59,65}. Similarly, recent studies suggest that the TP0750–TP0751 protease complex^{74,75} and TP0435⁷⁸ are expressed at low levels on the surfaces of some treponemes and, consequently, of limited availability to antibody binding. Two distinct mechanisms for modulating antibody reactivity with Tprs have been proposed. TprK undergoes sequence and antigenic variation in seven hypervariable regions, B-cell epitopes proposed to be located in external loops, through gene conversion from a donor site located elsewhere on the chromosome^{55,82–86}. Recent resequencing of the genome of strain SS14 has revealed sequence heterogeneity within the β -barrel-encoding portion of TprD (TP0131)⁸⁷, suggesting that this OMP also undergoes antigenic variation. A second mechanism pertains to TprE, TprG and TprJ; changes in the number of G nucleotide repeats immediately upstream of their transcriptional start sites, due to slipped strand mispairing during DNA replication, influences the amount of message produced⁸⁸. Unquestionably, much more investigation, including far better characterization of *T. pallidum*'s entire OMP repertoire and individual surface molecules, is needed before the pieces of this complex puzzle come together.

What lays beneath

Cell envelope structure

The architectural plan for the cell envelope of *T. pallidum* differs substantially from that of Gram-negatives⁸⁹. Images obtained by conventional transmission electron microscopy of fixed, embedded organisms showed the outer and cytoplasmic membranes juxtaposed with the peptidoglycan layer sandwiched between⁹⁰. However, visualization of the cell envelope in its native state by cryoelectron tomography^{15,91} revealed that the two membranes are actually well separated with a thin peptidoglycan layer creating two distinct zones within the periplasmic compartment (Fig. 1F). The lower, denser zone contains the periplasmic domains of integral proteins of the cytoplasmic membrane (various permeases), and the polypeptide moieties of lipoproteins, many of which are substrate-binding proteins (SBPs) for ABC transporters (Fig. 1E). Near the cell poles are chemoreceptor arrays containing the *T. pallidum*'s four methyl-accepting chemotaxis proteins (Mcp1 (TP0040), Mcp2 (TP0488), Mcp3 (TP0639) and Mcp4 (TP0640)) (Fig. 1G)⁹¹, sensors that bind exogenously derived ligands within the periplasm and relay chemotactic signals to the more distal flagellar motors⁹². A cone-like lattice at the cell tips, connected by fine fibrils to the outer membrane, could function as an organelle mediating end-on attachment (Fig. 1H)^{15,91}. The proximity of this tip structure to the flagellar motors and chemoreceptor arrays theoretically enables close coordination between attachment, environmental sensing and directed motility. By providing receptors distributed along the length of the cell body, the aforementioned adhesins would enable organisms to gain an initial 'foothold' before becoming stably attached at their tips.

Transport across the cytoplasmic membrane

T. pallidum uses diverse ABC transporters and symporters (totaling ~5% of its genome) to transfer molecules required for cell viability from periplasm to cytosol (Fig. 1E, also see TransportDB, <http://membranetransport.org/>). Following passive diffusion of glucose across

the outer membrane through nonselective porins, the methylgalactoside (Mgl) glucose-galactose ABC transporter (consisting of TP0545 and TP0684-0686) is believed to mediate high-affinity transport across the cytoplasmic membrane⁹³. Consistent with its lack of biosynthetic capacity, *T. pallidum* seems to possess a broad, though not easily understood, transporter repertoire for amino acids. One ABC transporter, MetI-MetN-MetQ (TP0119-TP0120-TP0821 (also known as Tpn32)) is dedicated to the uptake of methionine⁹⁴. The genome encodes three other putative SBPs with predicted specificities for oligopeptides (OppA (TP0585)), histidine (HisJ (TP0308)) and polar amino acids (TP0309), but their corresponding permeases and ATP-binding proteins have yet to be identified. Rounding out the *T. pallidum*'s amino acid requirements are putative symporters for aspartate and glutamate (TP0555 and TP0934), alanine and glycine (TP0414 and TP0998) and branched chain amino acids (TP0265). In addition, TP0144 functions as the SBP for a thiamine ABC transporter (TbpAPQ(TP0142-0144))⁹⁵. BioMNY (TP0226-0228), a putative biotin importer, seems to be the bacterium's only energy coupling factor (ECF)-type ABC transporter⁹⁶.

Structural biology has become an essential tool for studying cell envelope constituents of *T. pallidum*. The crystal structure of TroA (TP0034), originally thought to be a rare OMP, revealed that it belonged to a newly discovered class of SBPs associated with transition-metal ABC transporters⁹⁷. Crystallography and binding studies showed TmpC (TP0319) to be the SBP for the first ABC-type purine nucleoside transporter system (PnrABCDE (TP0319-323)) described in any bacterium⁹⁸. Another lipoprotein, TP0655, was shown to be the SBP for a polyamine transporter (PotABCD (TP0652-0655)) with nanomolar binding affinities for putrescine and spermidine⁹⁹. The X-ray structure of TP0298, also a lipoprotein, revealed it to be the SBP of a riboflavin transporter (RfuABCD (TP0298-0302))⁴¹ which collaborates with a dual function FAD pyrophosphatase-FMN transferase (Ftp (TP0796))^{42,100} to meet the *T. pallidum*'s prodigious requirements for flavin cofactors. A longstanding question has been how *T. pallidum*, a fatty acid auxotroph, obtains essential long-chain fatty acids (LCFAs). Recent structural analyses have led to the description of a multimeric lipoprotein complex (*TatT-TatP* (TP0956–TP0957)) thought to comprise a tetratricopeptide repeat (TPR)-protein associated transporter that traffics LCFAs across the periplasm^{101,102}.

Carbohydrate utilization and energy generation

Access to a plentiful supply of glucose in blood and interstitial fluids almost certainly explains why *T. pallidum* can rely on glycolysis as its primary means for generation of ATP (Fig. 2). In line with this notion is the lack of a complete pathway for gluconeogenesis and the inability to β -oxidize fatty acids and catabolize amino acids¹⁸. Telling in terms of the importance of glucose for driving *T. pallidum*'s 'engine' are classic studies showing rapid loss of motility upon glucose deprivation and rapid resumption of motility with it restored¹⁰³. Whether *T. pallidum* needs alternative carbon sources is unclear. In contrast to *Borrelia burgdorferi*, the Lyme disease spirochete¹⁰⁴, *T. pallidum* lacks genes for importing and utilizing exogenous glycerol, instead using glycerol phosphate dehydrogenase (GpsA (TP1009)) to convert dihydroxyacetone phosphate to glycerol-3-phosphate for phospholipid synthesis¹⁸. It also lacks phosphoenolpyruvate (PEP)-dependent phosphotransferase system

(PTS) permeases (a ubiquitous means among bacteria for importing and regulating uptake of alternative sugars¹⁰⁵), relying, instead, on hexokinase to phosphorylate glucose as the first step in glycolysis¹⁸. Bioinformatics, however, predict at least one additional ABC sugar permease (TP0075–TP0076), which uses two different SBPs (TP0074 and TP0737) and a nucleotide ATP-binding subunit, TP0804, an ortholog of *E. coli* MalK¹⁸. The functionality of this putative multiple sugar transporter is supported by the observation that, in addition to glucose, maltose and mannose can support replication of *T. pallidum in vitro*³⁵.

Some clever modifications in the conventional glycolytic pathway, shared with phylogenetically diverse organisms (eubacteria, protozoa, and plants¹⁰⁶), enable *T. pallidum* to extract as much energy as possible from substrate level phosphorylation while enhancing metabolic versatility (Fig. 2). To spare ATP, *T. pallidum* uses a phosphofructokinase employing pyrophosphate (PP_i-PPFK (TP0542)) instead of the ATP-dependent enzyme (ATP-PPFK)¹⁰⁷; *B. burgdorferi*, also glycolysis-dependent, uses an orthologous enzyme¹⁰⁸. Moreover, whereas ATP-PPFK is irreversible and a major point for allosteric regulation of standard glycolysis¹⁰⁹, PP_i-PPFK is reversible and non-allosteric¹⁰⁷. A second modification is the substitution of the reversible pyruvate phosphate dikinase (PPDK (TP0746)) for pyruvate kinase^{18,108}. When coupled to adenylate kinase (TP0595), PP_i-PPDK yields four ATPs per glucose compared with the two from pyruvate kinase in standard glycolysis¹¹⁰. The reversibility of the PPDK reaction, in concert with PEP carboxykinase (PEPCK (TP0122)), and oxaloacetate decarboxylase (OadA-OadB (TP0056–TP0057)), creates a cycle for inter-conversion of pyruvate, PEP and oxaloacetate. PEP likely is central for *T. pallidum* to sense and regulate carbon and nitrogen flux (Box 2). *T. pallidum* can use oxaloacetate for its limited amino acid metabolism through conversion to aspartate by aspartate aminotransferase (TpaAT (TP0223)), with glutamate as the amine donor, and subsequently to asparagine by asparagine synthetase (AsnA (TP0556)). *T. pallidum* produces another ATP by converting pyruvate to acetate by pyruvate-flavodoxin oxidoreductase (PFOR (TP0939)), acetate kinase (AckA (TP0476)) and phosphate acetyl transferase (Pta (TP0094)). Importantly, whereas the more common pyruvate dehydrogenase reduces NAD⁺ to NADH, the electrons released by PFOR during oxidative decarboxylation of pyruvate are transferred to a low potential single electron carrier, probably flavodoxin (TP0925), which, as noted below, may then transfer them to a newly discovered pathway for chemiosmotic energy conservation.

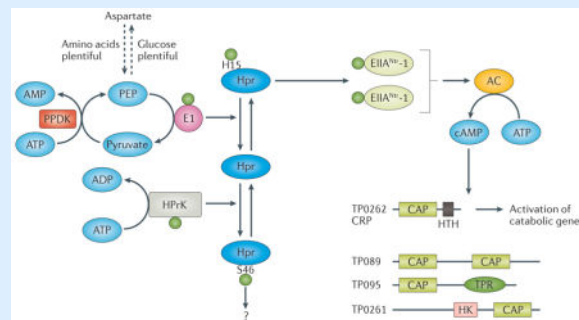
Box 2

A chimeric PTS for control of carbon and nitrogen metabolism

Although *T. pallidum* has access to a continuous supply of nutrients, their availability and proportions likely vary in different niches. The phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) appears to be the spirochete's primary mechanism for adjusting carbon and nitrogen utilization. Two general types of PTS have been described: a canonical PTS dedicated to carbohydrate utilization and a nitrogen-related PTS (PTS^{Ntr}) that is exclusively regulatory^{105,147}. Because *T. pallidum* lacks PTS permeases, its PTS must be solely regulatory. However, it is also chimeric, combining elements derived from the sugar PTSs of Gram-negatives (Enzyme I (EI (TP0575)) and histidine

phosphocarrier protein (Hpr (TP0589)) and Gram-positives (Hpr kinase (HprK (TP0591)) with the PTS^{Ntr}-derived components EIIA^{Ntr}-1 (TP0085) and EIIA^{Ntr}-2 (TP0755)^{105,148}. The resulting hybrid seems capable of intricate metabolic control. Although EI and HprK both phosphorylate Hpr, they do so at different sites: EI at His-15 (the catalytic site) and HprK at Ser-46 (the regulatory site), with PEP and ATP as the respective phosphoryl donors^{149,150}. Since phosphorylation at one site inhibits phosphorylation at the other, the activities of EI and HprK seem counter-regulatory. Recalling the inter-conversion of PEP and aspartate through oxaloacetate described earlier, one can envision how cross-talk between the EI and HprK-Hpr pathways might regulate carbon-nitrogen flux; our proposed scheme is based on Saier's postulate that the HprK-Hpr pathway is the direct regulator of glycolysis in treponemes¹⁵¹. When amino acids are relatively scarce, glycolysis would have to furnish substrate for their production in addition to meeting the cell's energy needs. The resulting drain on PEP would diminish phosphorylation of Hpr at His-15 by EI, leading to enhanced glycolysis driven by HprK-Hpr. When amino acids are plentiful, the flow would be reversed, and aspartate would be converted to PEP, promoting the EI pathway and antagonizing phosphorylation of Hpr at Ser-46 by HprK.

In Gram-positives, Hpr(Ser)-P activates catabolite control protein A (CcpA), the transcription factor responsible for carbon catabolite repression¹⁰⁵. *T. pallidum* does not contain this regulatory protein, so the effector molecule(s) influenced by the Hpr-HprK pathway is unknown. In *E. coli*, dephosphorylated EIIA^{Ntr} broadly influences metabolism by regulating intracellular potassium levels through its interactions with the potassium transporter Trk¹⁵², which *T. pallidum* possesses. Interacting partners of phosphorylated EIIA^{Ntr}s have not been identified in any bacterium¹⁵³. Given the elements *T. pallidum* has appropriated from the sugar PTS, one possibility is that one or both phosphorylated EIIA^{Ntr}s activate adenylate cyclase (TP0485)¹⁰⁵. Although admittedly without precedent, it is important to note that TP0485 belongs the largest and most diverse group of adenylate cyclases, Class III, whereas the extensively studied *E. coli* enzyme belongs to Class I¹⁵⁴. This has provocative implications given *T. pallidum*'s four predicted cAMP-binding proteins (TP0089, TP0261, TP0262, and TP0095) and the well-established link between production of cAMP and bacterial virulence¹⁵⁴.



Besides depriving *T. pallidum* of the full energy content of glucose, the absence of oxidative phosphorylation creates two metabolic quandaries: (i) how to regenerate NAD⁺ from NADH in order to maintain glycolysis and (ii) how to generate the electrochemical potential across the cytoplasmic membrane that is needed to drive the flagellar motor and nutrient uptake by

symporters. *T. pallidum* uses lactate dehydrogenase (LDH (TP0037)) and the flavoprotein NADH oxidase-2 (NOX-2 (TP0921)) to resolve the first dilemma (Fig. 2)^{111,112}. Under low oxygen conditions, such as on mucosal surfaces, conversion of pyruvate to lactate by LDH predominates. In aerobic environments, NOX-2 uses NADH as the electron donor to reduce molecular oxygen to water. Once in the presence of oxygen, lactate generated under anaerobic conditions can be reclaimed as pyruvate, with NOX-2 regenerating NAD⁺ from the resulting NADH. Thus, NOX-2 serves a dual purpose: it promotes production of acetate from pyruvate and protects against oxygen toxicity. Reliance on NOX-2 likely accounts for the observation that *T. pallidum*, once considered an anaerobe because of its exquisite sensitivity to ambient oxygen concentrations, actually requires 3–5% O₂ for optimal replication^{35,113}, values closely matching those within mammalian tissues¹¹⁴. To resolve the second dilemma, with the added dividend of boosting energy production, *T. pallidum* is believed to couple the generation of an electrochemical gradient by a non-canonical flavin-dependent *Rhodobacter nitrogen fixation* (Rnf) Na⁺/H⁺ redox pump to ATP synthesis by one or both of its A-type ATP synthases (formerly annotated as V-type ATPases) (Fig. 2)^{42,115}. Transfer of electrons from reduced flavodoxin, generated by PFOR, drives the electrogenic pump¹¹⁵ with NAD⁺, generated by LDH or NOX-2 depending upon oxygen availability, the ultimate electron acceptor.

Transition metals and redox stress

Transition metal uptake

Although lacking many well-characterized bacterial metalloproteins, genome mining and biochemical studies have clearly established that *T. pallidum* requires all three transition metals—(iron, manganese and zinc) to fulfill vital structural and catalytic functions^{116,117}; in fact, the recent identification of multiple Fe-S cluster proteins indicates a greater need for iron than previously recognized. Characteristically, *T. pallidum* seems to use arcane strategies to wage the battle for transition metals¹¹⁷. It cannot synthesize siderophores and does not possess a TonB ortholog for energizing transport across the outer membrane. It can extract iron from surface-bound lactoferrin and transferrin¹¹⁸, but the receptors are unknown. TpD (TP0971), a 34-kDa lipoprotein avidly binds human lactoferrin but is periplasmic¹¹⁹, and, therefore, cannot be functionally analogous to TbpB, the *Neisseria gonorrhoeae* lipoprotein co-receptor for lactoferrin¹²⁰. Ferriportin-mediated efflux of soluble Fe²⁺ from host cells,¹²¹ with subsequent diffusion across the outer membrane, might bypass the need for TonB-dependent uptake in some tissues. Whereas many pathogens possess highly redundant systems for transport of transition metals across the cytoplasmic membrane, *T. pallidum* seems to accomplish this task with just two ABC transporters: Tro (transport-related operon), which can import iron, manganese, and zinc, and the zinc-dedicated Znu^{116,122}. Import of iron across the cytoplasmic membrane may be complemented by TP0972, a member of the iron permease Ftr1 superfamily^{119,123,124}. A final metal-related oddity is *T. pallidum*'s ostensible lack of a global, transition metal-dependent transcriptional regulator. The *tro* operon encodes a zinc- and/or manganese-responsive DtxR-like repressor, TroR, which has no predicted binding sites outside of the *tro* promoter^{116,125}.

Dealing with oxidative stress

Because *T. pallidum* lacks many of the anti-oxidant enzymes common to other bacteria, its defenses against oxidative stress were long obscure, even with the availability of the genomic sequence. As information on novel anti-oxidant proteins in bacteria has grown in recent years, it has been possible to elucidate *T. pallidum*'s redox defense mechanisms^{117,126}. Instead of a classical superoxide dismutase, *T. pallidum* uses two iron-active center proteins, superoxide reductase (TP0823) and its reductant rubredoxin (TP0991), both acquired from hyperthermophilic anaerobes by a free-living ancestor,¹²⁷ to protect against O₂^{•-}. The presence of these and other iron-containing proteins creates the need to carefully regulate intracellular iron stores to minimize iron-catalyzed production of protein-damaging and DNA-damaging hydroxyl radicals¹²⁸. *T. pallidum* accomplishes this using the *dps* gene product TpF1 (TP1038), a dodecameric bacterioferritin with ferroxidase activity that binds soluble cytosolic Fe²⁺ and sequesters it as insoluble Fe³⁺¹²⁹. For defense against peroxides, the bacterium depends upon a single robust, broad-spectrum, NADPH-dependent peroxiredoxin, the flavoprotein alkyl hydroperoxide reductase C (TpAhpC (TP0509))¹³⁰. In other bacteria, AhpC-like peroxiredoxins are recycled by a dedicated flavoprotein disulfide reductase, alkyl hydroperoxide reductase F (AhpF). *T. pallidum* not only lacks this enzyme, its TpAhpC has diverged from other AhpCs to the extent that it cannot use AhpF as an electron donor; it relies instead on thioredoxin (TrX (TP0919)) and thioredoxin reductase (TrxR (TP0814))¹³⁰. Both TpAhpC and TpTrx are expressed at very high levels in spirochetes freshly harvested from rabbit testes¹³⁰, suggesting that peroxides are a major form of oxidative stress encountered during infection. Trx and TrxR presumably do double-duty defending against disulfide stress, although the low molecular weight, primary thiol redox buffer remains unidentified. Also unclear is how, without known redox regulatory proteins (for example, OxyR, SoxR, and PerR), the bacterium senses oxidative stress, although *in vitro* studies indicate it has the capacity to do so¹³⁰. The degree of disulfide bonding between TpF1¹³¹ and Tpp17¹³² monomers may serve as non-transcriptional means for redox sensing in the cytosol and periplasm, respectively.

Regulation of gene expression

Unlike most pathogens and all environmental bacteria, *T. pallidum* does not contain two-component systems that mediate large shifts in gene expression in response to alterations in growth conditions^{18,133}. On the other hand, in addition to a σ^{70} housekeeping sigma factor (RpoD (TP0493)), the *T. pallidum* genome encodes four annotated alternative sigma factors, σ^E (RpoE (TP0092)), σ^{54} (RpoN (TP1011)), σ^A (TP1012), and σ^{28} (RpoF (TP0700)), collectively indicating a capacity to re-direct gene transcription in response to specific environmental cues. While σ^{28} presumably transcribes flagellar genes, *T. pallidum* deviates from traditional flagellar biosynthesis pathways in that it lacks an ortholog for FlhDC, the master transcriptional regulator for class II flagellar genes, and its σ^{28} gene lacks an upstream binding site for σ^{54} ¹³⁴. Also notably absent is the alternative sigma factor (σ^{32}) that mobilizes a heat shock response, likely accounting for *T. pallidum*'s sensitivity to supra-physiologic temperatures¹³⁵. Furthermore, the genome encodes an unusual hybrid PTS system that putatively links carbon and nitrogen flux to gene regulation through the small nucleotide messenger cAMP (Box 2). The c-di-GMP signaling pathway is associated with a

wide range of adaptive processes and behaviors in other pathogenic bacteria¹³⁶. The presence of this pathway in *T. pallidum* is, therefore, a potentially important discovery. According to bioinformatics, *T. pallidum* contains two diguanylate cyclases for synthesizing c-di-GMP (TP0172 and TP0981)¹³⁶, three phosphodiesterases for degrading it (TP0764, TP0877, and TP0912), and a putative effector protein (TP0086), which contains a c-di-GMP-binding PilZ domain^{40,136}.

The susceptibility of the *T. pallidum* outer membrane to chemical and physical perturbations *in vitro*^{13,45} implies the need for a sensitive and robust system for sensing and responding to cell envelope stress *in vivo*. The σ^E (TP0092) pathway fulfills this function (Fig. 3A)¹³⁷. *tp0092* is highly transcribed during experimental syphilis,¹³⁸ suggesting that σ^E -dependent genes are strongly induced by the inflammatory milieu in the rabbit testis. *T. pallidum*'s pathway for regulation of σ^E includes DegS (TP0773), RseP (TP0600), and RseA (TP0093) orthologs¹³⁷. Notably absent is an identifiable ortholog for RseB, a negative regulator that binds to RseA and protects it from DegS-mediated cleavage¹³⁷.

To initiate transcription, members of the σ^{54} family of alternative sigma factors recognize a unique -24/-12 type promoter and require hydrolysis of ATP by an enhancer-binding protein (EBP) that binds to an upstream sequence^{139,140}. Further, interrogation of the *T. pallidum* genome for genes potentially transcribed by σ^{54} revealed *tpf1* as the strongest candidate σ^{54} -dependent gene¹⁸. The genome also encodes two EBPs, the nitrogen regulatory protein C (NtrC)-like TP0519 and the nitric oxide reductase regulator (NorR)-like TP0082 (Fig. 3B)¹⁸. TP1012, annotated as an ortholog of the Gram-positive housekeeping σ factor (σ^A)¹⁸, presents an interesting conundrum. It seems unlikely that *T. pallidum* would have two housekeeping sigma factors. However, TP1012 is also a distant relative of *E. coli* σ^S (RpoS); moreover, inspection of the *tp1012* sequence identified an ATG translational start with an excellently placed σ^{54} promoter. Thus, *T. pallidum* might harbor an EBP- σ^{54} - σ^S cascade (Fig. 3B) mirroring the virulence-related Rrp2- σ^{54} - σ^S pathway in *B. burgdorferi*^{141,142}.

Comparative Genomics

The overall level of genetic identity among the human pathogenic treponemes, $\geq 99.8\%$, implies that differences in virulence, invasiveness, and tissue tropisms arise from changes in a relative handful of genetic loci^{5,143,144}. Table 1 lists the strongest candidate proteins potentially responsible for phenotypic differences between *pallidum* and *pertenue* subspecies. Of the 22 genes listed, 15 encode proteins known or believed to reside in the cell envelope, with members of the Tpr family particularly well represented. Many of the amino acid substitutions in the Tprs are located in the C-terminal domains known or predicted to form outer membrane-inserted β -barrels (Fig. 4). Strikingly, the *tprA* and *tprF* loci, which typically encode frame-shifted truncated proteins in venereal syphilis spirochetes, encode full-length proteins in *pertenue* strains^{63,143,145}; the *pertenue* TprF β -barrel is TprI-like whereas the TprA barrel is unique (Fig. 4). The list in Table 1 also contains most of the candidates for differences between subspecies *pallidum* strains. The Tpr repertoires among venereal syphilis spirochetes (Fig. 4) are, on the whole, very similar, although sequence variation in known or potential β -barrel forming domains does occur (*e.g.*, TprC, TprD, and TprI), along with insertions (*e.g.*, TprG and TprJ), sporadic truncations (*e.g.*, TprG in

Seattle 81–4) or replacement of typically truncated proteins with full-length paralogs (for example, TprA, also in Seattle 81–4)¹⁴⁵.

Structural homology modeling of BamA has afforded valuable insights into sequence differences revealed by genomics⁶⁵. Mexico A-like strains have BamA β -barrels in which a single amino acid substitution in the immunodominant L4 surface loop markedly reduces reactivity of sera from patients infected with strains containing Nichols-like β -barrels^{65,146}. Petrosova *et al.*¹⁴⁶ made the seminal observation that the β -barrel in Mexico A TP0326 is identical to that in *pertenue* strains. They proposed that the *pertenue* TP0326 β -barrel was introduced into the subsp. *pallidum* genome as a result of recombination in an individual co-infected with yaws and venereal syphilis. Since this L4 epitope is a prime opsonic target⁶⁵, the resulting strain theoretically would be less susceptible to pre-existing Nichols anti-L4 antibodies and, thus, capable of spreading in populations in which Nichols-like strains predominate.

Concluding remarks

Winston Churchill's description of the Soviet Union as "a riddle, wrapped in a mystery, inside an enigma" could easily have been applied to *T. pallidum*. For decades, investigators obsessed with understanding protective immunity in syphilis pursued their Holy Grail, a syphilis vaccine, but were stymied by the 'black box' of the spirochete's surface. Syphilologists now have in hand authentic OMPs and the experimental tools needed to establish authenticity for other candidates. These proteins also will enable clarification of the immunologic cat and mouse game that characterizes human syphilis and formulation of strategies to tease apart the virulence properties of the pathogenic treponemes. A relatively recent development in syphilis pathogenesis research has been the recognition that stealth pathogenicity has complicated and surprisingly obscure underpinnings that warrant intensive investigation. This Review underscores a theme now taking shape: the spirochete's genomic reductionism, rather than being simply a matter of jettisoning unneeded genes, stems from a far more complicated evolutionary process, combining ultrastructural and metabolic parsimony with regulatory intricacy. Two forces appear to be at work: one is the need for *T. pallidum* to make the most of the nutrients it can usurp from its host, the other is the bacterium's need to adapt to a diversity of micro-environments and stresses encountered during its journey within the human body. One of the great challenges to understanding these forces will be developing strategies to move beyond the static picture that has been an inadvertent byproduct of studying spirochetes extracted from a single milieu — the inflamed rabbit testis — to one that is dynamic and integrative.

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Glossary terms

Toll-like receptor (TLR) 2

A pattern recognition receptor that recognizes a variety of pathogen-associated molecular patterns, including bacterial lipoproteins.

Opsonic antibodies

Antibodies directed against a pathogen's surface-exposed epitopes that bind to Fc receptors on a phagocytic cell, triggering internalization through phagocytosis.

Permease

A polytopic integral membrane protein that mediates energy-dependent uptake of small molecules across the plasma membrane of Gram-positives and the cytoplasmic membrane of Gram-negative bacteria.

ABC Transporter

An ATP-binding cassette transporter couples hydrolysis of ATP to transport (usually import) of a substrate across the cytoplasmic membrane of Gram-negative bacteria and the plasma membrane of Gram-positive bacteria. Classical bacterial ABC transporters have a modular composition consisting of a substrate-binding protein, a dimeric membrane-bound permease, and a dimeric nucleotide binding protein with ATPase activity.

Symporter

Transporter molecules that use the sodium or electrochemical gradient across the cytoplasmic membrane to drive the co-directional importation of substrates from the periplasmic to the cytosolic compartment.

Auxotroph

An organism that has lost the ability to synthesize molecules required for growth. *T. pallidum* is considered an extreme auxotroph because of its very limited biosynthetic capacity.

Two-component system

Two-component systems typically consist of a membrane-bound histidine kinase that senses a specific environmental stimulus and a cognate response regulator that mediates a cellular response, usually by activating and/or repressing differentially expressed genes.

Housekeeping sigma factor

A sigma factor that binds to the catalytic core of RNA polymerase and recognizes promoters of genes required for core bacterial cell functions, such as maintenance and metabolism.

Alternative sigma factor

A sigma factor that binds to the catalytic core of RNA polymerase, displacing the housekeeping sigma factor, re-directing transcription towards genes required to respond to a particular environmental stimulus, condition, or stress.

Heat-shock response

The bacterial cell's response to a sudden increase in temperature, involving differential gene expression regulated through the alternative sigma factor σ^{32} .

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Key points

- Pathogenic treponemes are clonal, uncultivable, highly invasive bacteria that cause venereal syphilis, yaws, endemic syphilis, and pinta—multi-stage, infections that have many similarities but can be differentiated based on clinical, epidemiologic, and geographic criteria. Only *Treponema pallidum* subsp. *pallidum* is transmitted by sexual activity.
- Key to the syphilis spirochete's capacity for immune evasion and thus 'stealth pathogenicity' is its unusual outer membrane, which lacks lipopolysaccharide and contains an extremely low density of integral membrane proteins and a paucity of surface-exposed lipoproteins. Production of opsonic antibodies against low abundance surface antigenic targets is believed to be essential for control of syphilitic infection.
- In recent years, considerable progress has been made in defining the syphilis spirochete's repertoire of β -barrel-forming rare outer membrane proteins and the mechanisms by which the bacterium seems to limit exposure of surface molecules to the host's antibody-mediated defenses.
- During the course of genomic reduction, *T. pallidum* has undergone adaptations that enable it to acquire all of its required nutrients from its obligate human host and optimize their usage within various niches, while coping with exogenous and endogenous stress.
- The *T. pallidum* genome encodes several alternative sigma factors and other regulatory molecules/pathways that collectively point to a previously unsuspected capacity to intricately regulate gene expression within diverse microenvironments.
- Comparative genomics has enabled investigators to identify 'hot spots' for sequence variation that likely explain differences in virulence potential and tissue tropisms among the pathogenic treponemes; many of these are located in proteins known or predicted to reside at the host-pathogen interface.

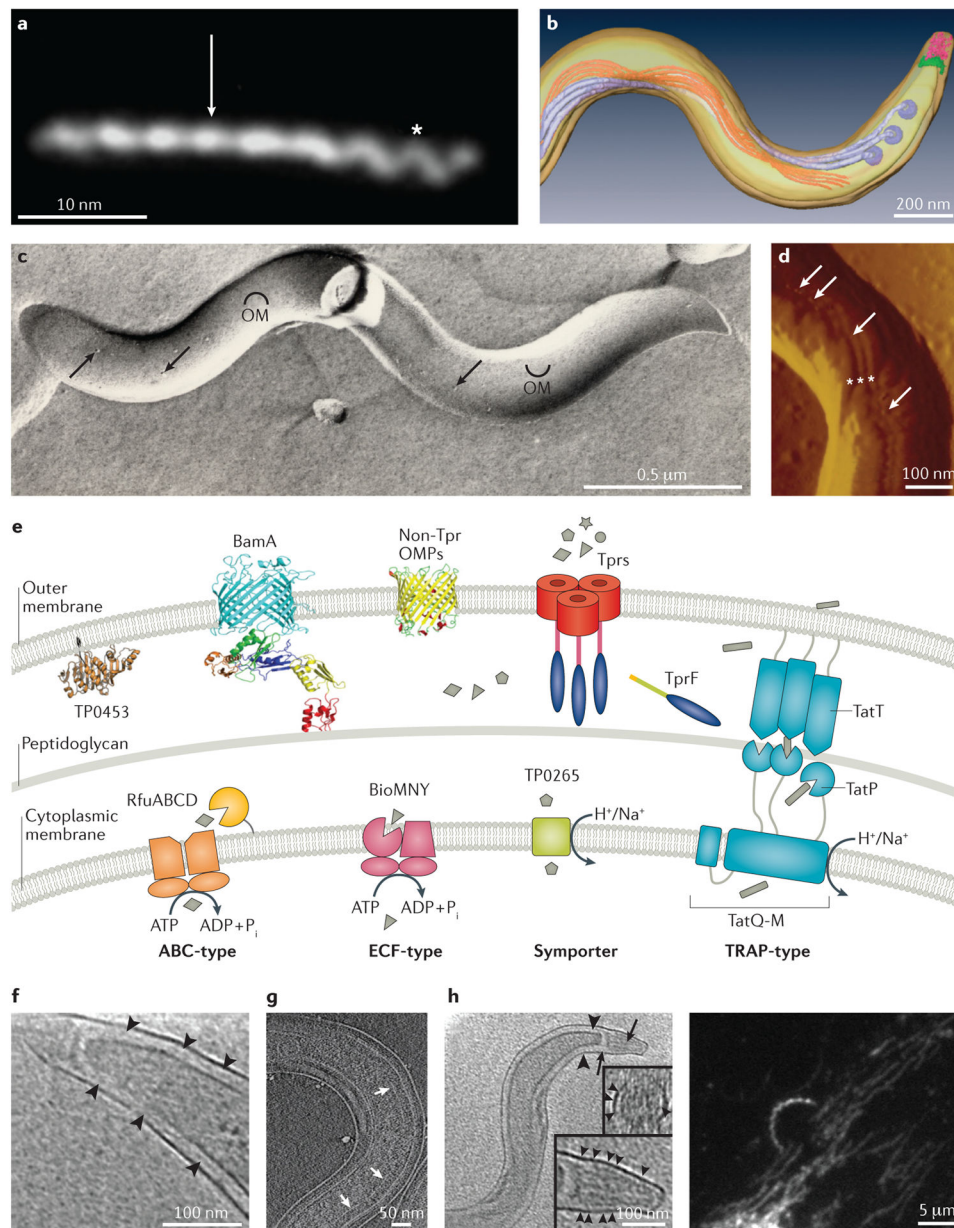


Figure 1. Morphology and cell envelope architecture of *T. pallidum*, the stealth pathogen
A. Darkfield micrograph showing the flat wave morphology of *T. pallidum*. Asterisks and arrowhead indicate segments oriented 90 degrees from each other (with permission from Reference 15). **B.** Top view of a surface-rendered model of *T. pallidum* generated from cryoelectron tomograms showing the outer and cytoplasmic membranes (transparent yellow), flagellar motors (basal bodies, dark lavender), flagellar filaments (light lavender), cytoplasmic filaments (orange), cap (green), and cone (pink) (with permission from Reference 15). **C.** Freeze-fracture electron microscopy reveals scarce particles (integral membrane proteins) within the *T. pallidum* outer membrane. Convex and concave leaflets of the outer membrane are indicated; arrowheads indicate particles in the two leaflets (with permission from Reference 155). **D.** Scanning probe microscopy reveals rare particles on the

T. pallidum surface (arrows), often located on the bulge in the outer membrane created by the underlying periplasmic flagella (asterisks) (with permission from Reference 91). **E.** Model for the molecular architecture of the *T. pallidum* cell envelope. Shown in the outer membrane are BamA (TP0326)^{59,64}, a generic bipartite (that is, full-length) Tpr attached by its N-terminal portion to the peptidoglycan^{59–61}, a generic non-Tpr β -barrel, and TP0453 (p30.5), a structurally characterized lipoprotein attached to the outer membrane inner leaflet¹⁵⁶. Tprs, such as TprF, lacking the MOSPC β -barrel forming domain, are located in the periplasm⁵⁹. Substrates present in high concentration in the extracellular milieu probably traverse the outer membrane by simple diffusion through porins, such as TprC. Prototypic ABC-like transporters use a periplasmic substrate-binding protein (SBP), typically lipoproteins, and components with transmembrane and ATP-binding domains. The energy coupling factor (ECF)-type ABC transporters uses a transmembrane ligand-binding protein in place of a separate periplasmic SBP. To substitute for ATP hydrolysis, the symporters use a transmembrane permease that relies on energy from a chemiosmotic or electrochemical gradient that may be generated by the Rnf complex. The tripartite ATP-independent periplasmic (TRAP) transporters also lack ATP-binding modules and use transmembrane electrochemical gradients, but they are more complex. *T. pallidum* seems to have evolved a variation on the TRAP theme by also using an additional periplasmic component protein (TatT (TP0956)) containing a tetratricopeptide repeat (TPR) motif, giving rise to a newly described system denoted as a TPR protein-associated TRAP transporter (TPAT). The TPR protein TatT likely associates with the SBP TatP (TP0957) in a heterohexameric fashion to carry out ligand binding and uptake; structural analyses suggest that this complex may accommodate a chain-like hydrophobic molecule(s), such as a long-chain fatty acid(s). Uptake likely is facilitated by a putative membrane permease (TatQ-M (TP0958)) of the TPAT system^{101,102}. **F.** Cryotomographic section of *T. pallidum* near the cell end showing the peptidoglycan layer (arrowheads) midway between the outer membrane and cytoplasmic membrane (with permission from Reference 15). **G.** Cryotomographic section of *T. pallidum* showing chemoreceptor arrays (arrows, with permission from Reference 91). **H.** Cryotomographic slice showing the cone-shaped structure at *T. pallidum* cell ends (with permission from Reference 15) along with darkfield micrograph of *T. pallidum* stably attached via its tip to the surface of a trophoblast cell. White arrowheads indicate fine fibers between the cone and the outer membrane.

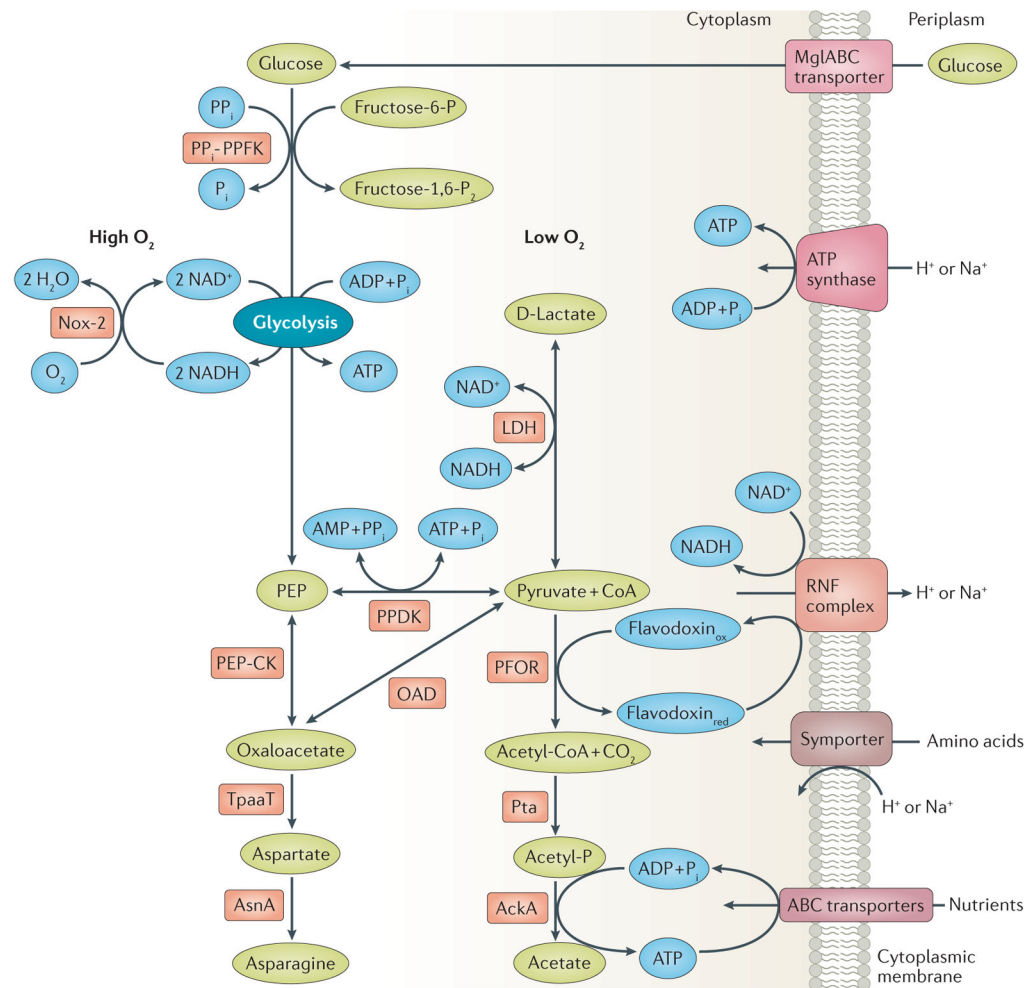


Figure 2. Energy generation, amino acid biosynthesis, and regeneration of NAD⁺ in *T. pallidum*
T. pallidum's glycolytic and pyruvate-to-acetate fermentation pathways are coupled to a putative Rnf pump, resulting in production of ATP by an ATP synthase; phosphoenolpyruvate (PEP) from glycolysis can be further metabolized to amino acids via PEP carboxykinase (PEP-CK). Metabolites, enzymes, and cofactors are shown in green, red, and blue, respectively. Abbreviations for enzymes are as follows: PPFK, phosphofructokinase; Nox, NADH oxidase; PPK, pyruvate phosphate dikinase; OAD, oxaloacetate decarboxylase; TpaAT, aspartate aminotransferase; AsnA, asparagine synthetase; LDH, D-lactate dehydrogenase; PFOR, pyruvate-flavodoxin oxidoreductase; Pta, phosphate acetyl transferase; AckA, acetate kinase. Abbreviations for compounds/metabolites are as follows: Fru-6-P, fructose 6-phosphate; Fru-1, 6-P₂, fructose 1, 6-bisphosphate; Pi, inorganic phosphate; PPi, inorganic pyrophosphate; CoA, coenzyme A; Acetyl-P, acetyl phosphate; RNF, a putative chemiosmotic pump with similarities to the *Rhodobacter nitrogen fixation complex*; Flavodoxin_{ox} and Flavodoxin_{red}, oxidized and reduced forms of flavodoxin.

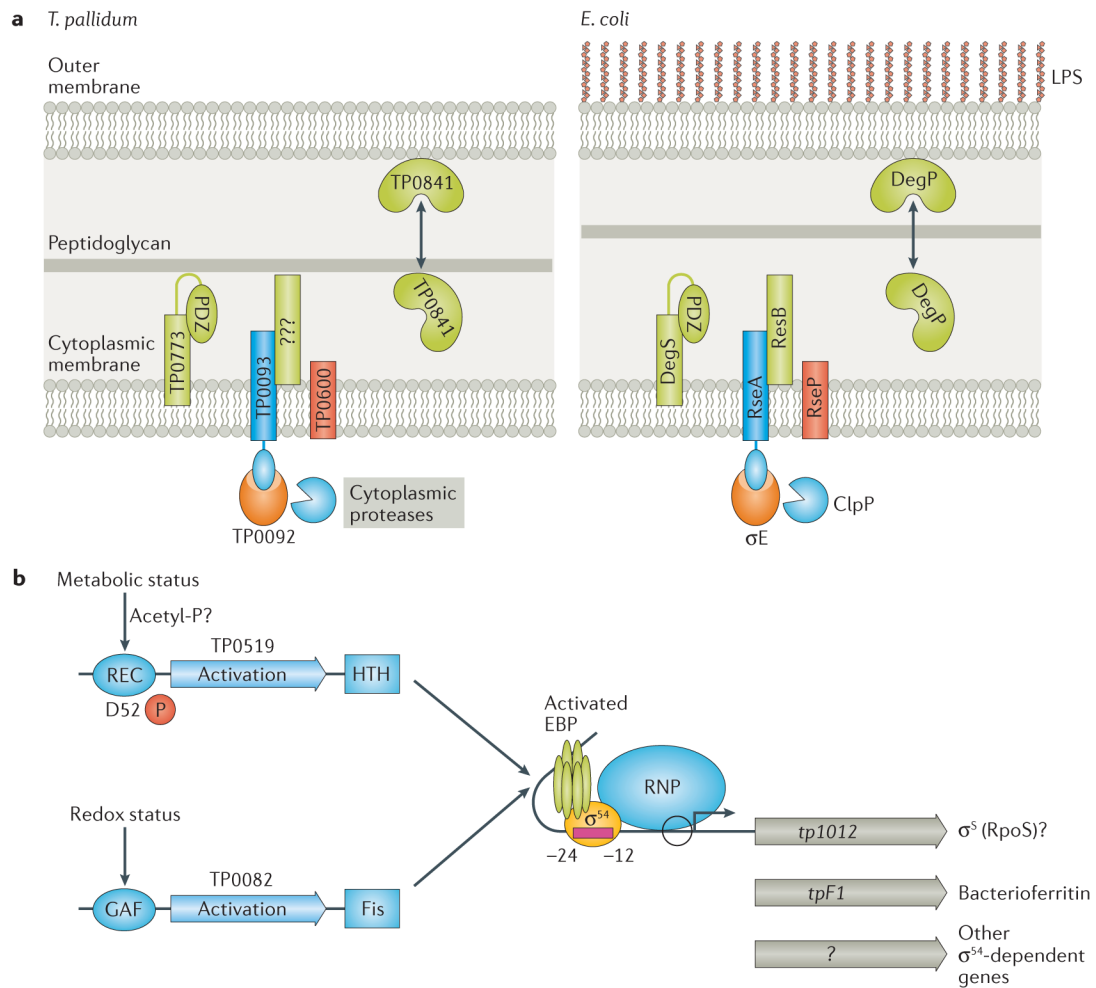


Figure 3. Proposed pathways for control of alternative sigma factors in *T. pallidum*

A. σ^E cell envelope stress responses in *T. pallidum* and *E. coli*. Binding of misfolded OMPs to the PDZ domain of DegS (TP0773) initiates transduction of the stress signal across the cytoplasmic membrane. *T. pallidum* lacks a recognizable ortholog for RseB, a negative regulator that binds to RseA and protects it from DegS-mediated cleavage. **B.** σ^{54} (RpoN)-dependent gene expression. *T. pallidum* contains two enhancer binding proteins, the NtrC-like TP0519 and the NorR-like TP0082. Because TP0519 lacks a cognate histidine kinase, phosphorylation of TP0519 may occur through an acetyl-phosphate donor. Activation of TP0082 is predicted to occur following the binding of a small molecule to its GAF domain. *tpF1* (*tp1038*), encoding a bacterioferritin, and *tp1012*, encoding an RpoS-like sigma factor, are predicted to be transcribed by σ^{54} .

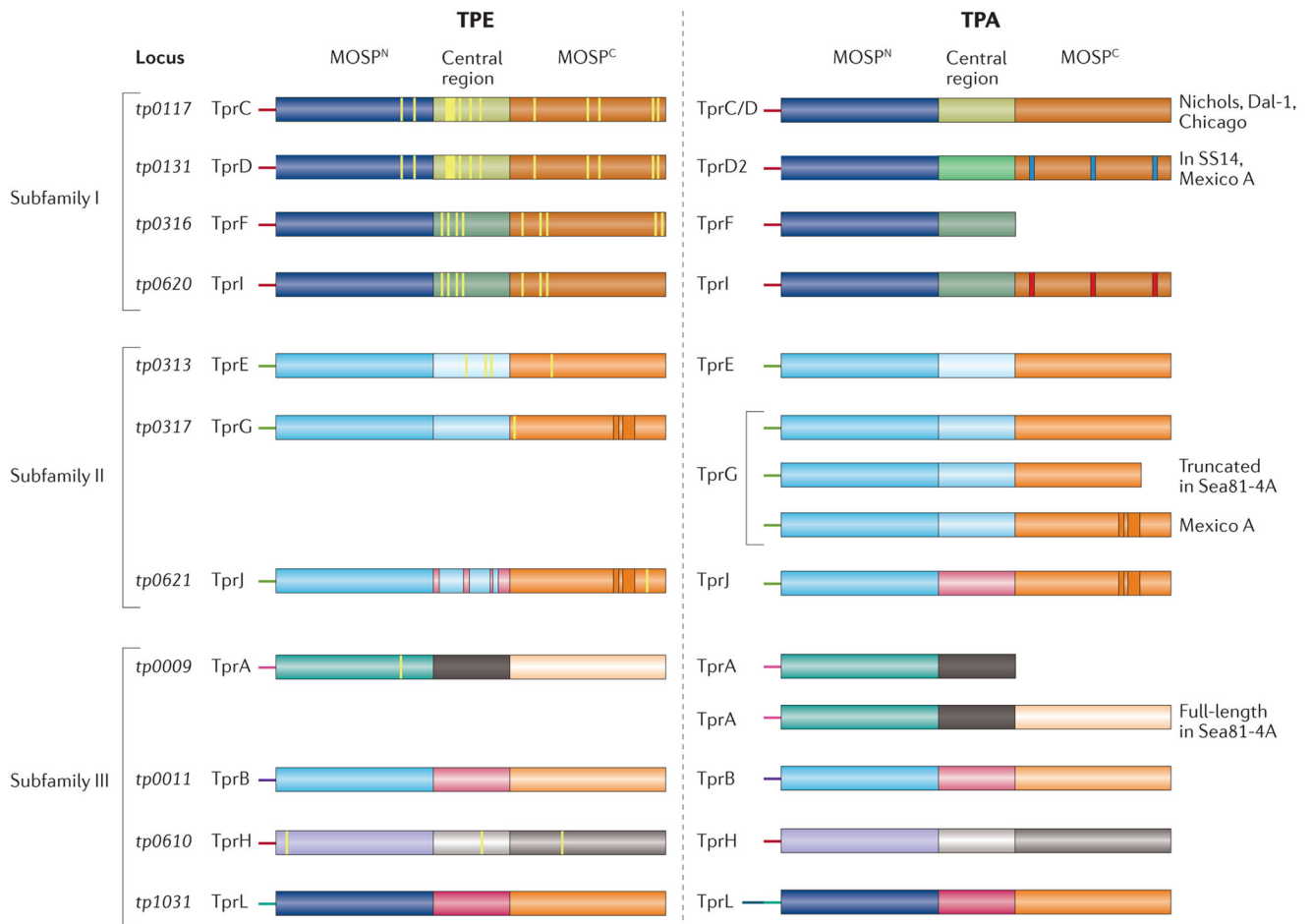


Figure 4. Domain architecture of the *T. pallidum* repeat (Tpr) family of proteins Tprs in *T. pertenuis* (TPE) and *T. pallidum* (TPA) strains

The Tprs are divided into three subfamilies based on sequence relatedness; within subfamilies I and II, members are closely related to each other, whereas members of subfamily III are heterogeneous^{52,63}. Full-length Tprs consist of: (i) a variable extreme N-terminal stretch (~50 amino acids); (ii) MOSP^N, a conserved N-terminal domain related to the corresponding domain in the N-terminus of the major outer sheath protein (MOSP) of *T. denticola*; (iii) a variable central region; and (iv) MOSP^C, a conserved C-terminal β -forming domain related to the corresponding domain in *T. denticola* MOSP. The thin yellow lines denote amino acid variation in TPE paralogs relative to the corresponding paralogs in the TPA Nichols strain. Shown in umber are insertions in the MOSP^C domains of TprG and TprJ relative to the corresponding paralog in the TPA Nichols reference strain. The central domain of TprJ in TPE contains sequences derived from the corresponding region of TprG. TprL in the TPA Nichols strain contains an extended variable N-terminal stretch. In TPE, TprA and TprF are full-length, bipartite proteins. The blue and red lines, respectively, are used to indicate sequence variation in TprD2 and TprI relative to TprC/D in TPA Nichols. With the exception of the Sea81-4 TPA strain, TprA is truncated, lacking the MOSP^C

domain; TprF is truncated in all TPA strains. TprK is not shown because of its hypervariability.

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Table 1

Candidate proteins potentially responsible for phenotypic differences between *T. pallidum* subsp. *pertenue* (TPE) and subsp. *pallidum* (TPA) strains*

Protein	Name	Predicted function and comments	References
TP0009	TprA	Potential porin (when C-terminal domain is present)	59–61
TP0117**	TprC	Porin	59,60
TP0131**	TprD	Porin	59,60
TP0133		HP, immunogenic protein	157,158
TP0134		HP, putative outer membrane protein	Annotation
TP0136**		Fibronectin-binding protein	76,77
TP0314		HP, not annotated in TPA	Annotation
TP0316	TprF	Potential porin (when C-terminal domain is present)	59,60
TP0326**	BamA	Originally Tp92; Outer membrane biogenesis	58,64,65
TP0433**	Arp	Acidic repeat protein, function unknown	Annotation
TP0462**		HP, putative lipoprotein	Annotation
TP0488**	Mcp2-1	Methyl-accepting chemotaxis protein	Annotation
TP0548**		Predicted rare outer membrane protein	57
TP0619**		HP	Annotation
TP0620**	TprI	Porin	59,60
TP0621	TprJ	Potential porin	59–61
TP0733		HP, outer membrane protein	Annotation
TP0858		Predicted outer membrane protein	57
TP0865**		HP, putative outer membrane protein	Annotation
TP0897**	TprK	Conflicting evidence regarding status as a <i>bona fide</i> outer membrane protein (see text)	52,56,61
TP0968		HP	Annotation
TP1031**	TprL	Potential porin	59–61

* Proteins with six or more amino acid replacements and/or major sequence differences between TPE and TPA strains according to [ejková et al.](#)¹⁴³

** Candidates for phenotypic differences between TPA strains (Nichols, DAL-1, Chicago, SS14, and MexicoA).

HP, hypothetical protein.