Published in final edited form as:

Mol Microbiol. 2009 June ; 72(5): 1081–1086. doi:10.1111/j.1365-2958.2009.06711.x.

***Treponema pallidum*, the Stealth Pathogen, Doth Change, But How?**

Justin D. Radolf^{1,2,*} and Daniel C. Desrosiers¹

¹Department of Medicine, University of Connecticut Health Centre, Farmington, CT 06030-3715

²Department of Genetics and Development, University of Connecticut Health Centre, Farmington, CT 06030-3715

Summary

Treponema pallidum rapidly disseminates from a genital site of inoculation to diverse organs where it establishes persistent infection. *T. pallidum* has long been regarded as a stealth pathogen because of its poorly antigenic and non-inflammatory surface. There is now increasing evidence that antigenic variation also contributes to the ability of the spirochete to evade host defences. Among the small number of proteins encoded by the *T. pallidum* genome with sequence similarity to well characterized transcription factors is TP0262, an orthologue for cAMP regulatory protein (CRP) of *E. coli*. Giacani and co-workers identified sequences matching the CRP consensus-binding motif upstream of the promoters of *tprE*, *tprG*, and *tprJ*, three members of the *T. pallidum* repeat (*tpr*) gene family (Subfamily II). Using EMSA, DNaseI footprinting, and an *E. coli*-based reporter system, they demonstrated that TP0262 specifically recognizes the putative binding sequences and that DNA binding is cAMP-dependent. Their report, a major methodological advance for syphilis research, suggests that *T. pallidum* has appropriated a paradigmatic global regulator of metabolic processes in heterotrophic bacteria to further its capacity for immune evasion in its obligate human host.

Key words/phrases

Treponema pallidum; syphilis; *tpr* genes; gene regulation; cAMP regulatory protein; carbon catabolite repression

Treponema pallidum, the causative agent of syphilis, is a sexually transmitted spirochaete that rapidly disseminates from a site of inoculation in the genital region to diverse organs where it can establish persistent, even lifelong, infection (Lukehart, 2004). The remarkable ability of this extracellular bacterium to evade host clearance mechanisms has earned it the designation “stealth pathogen” (Radolf *et al.*, 2006). Efforts pre-dating the genomic era to uncover the basis for *T. pallidum* stealth pathogenicity have focused on the unusual molecular architecture of its outer membrane, a lipid bilayer with a paucity of integral-membrane proteins that is non-inflammatory and presents few surface antigenic targets to the host immune system (Radolf, 1995). Since the publication of the *T. pallidum* genomic sequence (Fraser *et al.*, 1998), evidence has mounted that a protein-deficient outer membrane is only one facet of the persistence strategy of this spirochaete. *T. pallidum* may possess a complex system for antigenic variation to further confound host antibody responses (Centurion-Lara, 2006; LaFond and Lukehart, 2006). *T. pallidum* also must acquire nutrients in every microenvironment in which it takes up residence and, like all bacteria, must be able to sense and respond to changes in nutrient

* Address correspondence to: Justin D. Radolf, M.D., Department of Medicine, University of Connecticut Health Centre, 263 Farmington Avenue, Farmington, CT 06030-3715, Phone: 860 679-8480, FAX: 860 679-1358, JRadolf@up.uchc.edu.

availability as well as other environmental stresses (Hazlett *et al.*, 2002; Desrosiers *et al.*, 2007). Delineating virulence-related and physiologic processes, their interrelationships, and the regulatory networks that orchestrate them at transcriptional and post-transcriptional levels is a daunting challenge with a bacterium that can neither be cultivated nor manipulated genetically. The report by Giacani *et al.* (Giacani *et al.*, 2009) in this issue of Molecular Microbiology represents a step along the arduous path of developing the surrogate systems needed to elucidate gene regulation in *T. pallidum*. Their finding that *T. pallidum* might have appropriated CRP (cAMP regulatory protein), the paradigmatic global transcriptional regulator of metabolism in Gram-negative bacteria, for the purpose of immune evasion represents an unanticipated convergence of ostensibly unrelated themes in syphilis pathogenesis research.

When more than one carbohydrate is present in the growth medium, free-living heterotrophic bacteria will typically utilize a preferred carbon source while repressing uptake of less desirable, alternative substrates until the primary source is depleted. The regulatory processes by which bacteria prevent utilization of secondary carbon sources, collectively called carbon catabolite repression (CCR), have been intensively studied in *Escherichia coli* and *Bacillus subtilis* (Fig. 1)(Gorke and Stulke, 2008; Warner and Lolkema, 2003). In both model organisms, CCR centres about the multi-component phosphoenolpyruvate: sugar phosphotransferase (PTS) system for uptake and phosphorylation of sugars but employs markedly dissimilar mechanisms to achieve similar regulatory outcomes. In *E. coli*, the ratios of phosphoenolpyruvate to pyruvate, reflective of glucose consumption in glycolysis, determine the phosphorylation state of the EIIA component of the PTS. EIIA is preferentially dephosphorylated when *E. coli* cells grow in the presence of phosphate-draining, rapidly metabolizable sugars (e.g., glucose); unphosphorylated EIIA allosterically inhibits non-PTS sugar permeases. Phosphorylation of EIIA, which predominates when glucose is unavailable, activates membrane-bound adenylate cyclase. At sufficiently high levels of cAMP, CRP becomes activated and binds to specific DNA sequences at or near the promoters of genes encoding transporters and enzymes required for utilization of alternative carbon sources. In *B. subtilis*, histidine protein (Hpr) and Hpr kinase, (HprK), rather than EIIA, are the key regulators. HprK is activated by glycolytic intermediates, such as fructose-1–6-P, whereupon, at the expense of ATP, whereupon it phosphorylates Ser46 in the “activation site” of Hpr. Hpr (Ser-P) complexes with CcpA (catabolite control protein A), which then binds to *cre* sites of catabolic genes, repressing their transcription. As in *E. coli*, phosphorylation of Hpr at the “catalytic site”, His15, by EI (PtsI) of the PTS system promotes phosphorylation of EIIA, stimulating PTS-mediated transport and phosphorylation of glucose. In *B. subtilis*, Hpr(His-P) also negatively regulates both glycerol kinase (GlpK) and transcriptional regulators of alternative source PTS permeases. An important distinction between the two schemes is that regulation in *E. coli* is linked directly to PTS function via EIIA, whereas in *B. subtilis*, the linkage is indirect (Fig. 1). It is theoretically possible, therefore, for HprK/Hpr to regulate metabolic processes independent of the PTS system. Because pathogenicity programs in bacteria are often tied to the accessibility of specific nutrients associated with a particular host niche, it should not be surprising that there are now numerous examples in which CCR-mediated responses are involved in the coordination of virulence expression (Gorke and Stulke, 2008).

Consistent with its enigmatic evolutionary route (Norris and Weinstock, 2006), *T. pallidum* harbours an odd assortment of CCR-related molecules that does not conform to either the Gram-negative or Gram-positive paradigms. It contains Hpr (TP0589) and HprK (TP0591), as well as a pseudogene encoding a nonfunctional EI (TP0575), but does not have PTS permeases (Gonzalez *et al.*, 2005). In an elegant biochemical analysis using purified recombinant *T. denticola* (Td) Hpr and HprK orthologues, Gonzalez and colleagues (Gonzalez *et al.*, 2005) showed that Td HprK is capable of phosphorylating Hpr (presumably at Ser46) and is activated by 1–6 fructose bis-phosphate and gluconate-6-P. They speculated that in both *T. pallidum* and

T. denticola, Hpr/HprK exerts a regulatory function divorced from PTS sugar transport and phosphorylation. Curiously absent from this signalling mechanism, however, is a downstream regulatory target, because both treponemes lack CcpA. The presence in *T. pallidum* of a well-developed cAMP signalling system without PTS permeases deepens the conundrum. Surprisingly, the spirochaete contains not only adenylate cyclase (TP0485) but also four proteins (TP0089, TP0261, TP0262, and TP0995) with cAMP binding domains (Subramanian *et al.*, 2000; Giacani *et al.*, 2009). Of the latter, only TP0262 has extensive sequence homology with *E. coli* CRP (Fig. 2A). Homology modelling confirms that TP0262 and CRP are close structural orthologues (Fig. 2B). Giacani *et al.* (Giacani *et al.*, 2009) took note of the high degree of similarity between CRP and TP0262 and used it as the starting point for their ground breaking study (see below).

Although glucose is the primary energy source for *T. pallidum*, it also contains ABC transporters thought to be collectively capable of importing multiple sugars and, in an *in vitro* tissue culture system, this bacterium can metabolize mannose and maltose in addition to glucose (Gonzalez *et al.*, 2005) (Fraser *et al.*, 1998; Cox and Radolf, 2006). These data imply that, under some circumstances, *T. pallidum* can turn to alternative substrates to generate ATP and that, conversely, when glucose is readily available, it would have need for some form of CCR to modulate import of less desirable substrates. From the above, one would expect HprK/Hpr, rather than TP0262, to be the primary regulator of this putative CCR mechanism. Liberated from its conventional physiologic role, TP0262 might then have evolved to coordinate responses of more functionally divergent target genes and also to engage in cross-talk with other members of the treponemal cyclic nucleotide signalling family. Although many features of these novel signal transduction pathways and their potential points of intersection are difficult to discern, one might surmise that they collectively endow the spirochaete with a capacity for sensing and responding to environmental cues that could come into play at different sites and stages of syphilitic infection.

Given the dearth of recognizable transcription factors in *T. pallidum* (Fraser *et al.*, 1998; Subramanian *et al.*, 2000), TP0262 is an attractive starting point for an examination of global gene regulation in *T. pallidum* despite or, as reasoned by Giacani and co-workers (Giacani *et al.*, 2009), because of its possible “orphan” status as a metabolic regulator. They began by using the powerful tool of the modern molecular biologist, bioinformatics, to scan the *T. pallidum* genome for matches to the *E. coli* CRP consensus binding motif. Of the more than 200 hits obtained (but, unfortunately, not presented in tabular form), the ones of immediate interest were upstream of *tprE*, *tprG*, and *tprJ*, subfamily II in the 12 member *tpr* (*T. pallidum* repeat) gene family. The Tpr protein family has come under intense scrutiny since its discovery a decade ago by this same group (Centurion-Lara *et al.*, 1999) and others (Fraser *et al.*, 1998). Tprs are candidate rare outer membrane proteins that, by loose analogy with the systems for antigenic variation in the relapsing fever and Lyme disease spirochaetes (Norris, 2006), have been proposed to endow *T. pallidum* with the ability to alter its surface antigenicity (LaFond and Lukehart, 2006). Subfamily II genes have drawn interest of late because they are variably expressed in different *T. pallidum* clinical isolates and have hypervariable homopolymeric guanosine tracts near their transcriptional start sites that may be involved in their transcriptional control (Giacani *et al.*, 2007). The *in silico* results were confirmed in electrophoretic mobility shift assay (EMSA) and DNaseI footprinting using purified recombinant TP0262. Using a recently devised *E. coli*-based reporter system (Giacani *et al.*, 2007), expression of TP0262 was shown to modulate the promoter activities of the three genes. Lastly, in both EMSA and *E. coli*, the DNA-binding activity of TP0262 was cAMP-dependent. Based on prior mapping of transcriptional start sites (Giacani *et al.*, 2005), the authors propose that CRP modulates transcription of the three *tpr* genes, in concert with the polyG tracts, via interactions with σ^{70} . It is interesting to note, however, that the binding sites for all three genes are considerably further upstream from their respective promoter elements than are the sites

for canonical CRP-dependent promoters (Busby and Ebright, 1999), though within the extended range observed in genome-wide surveys of CRP-dependent genes (Zheng *et al.*, 2004; Gosset *et al.*, 2004). Resolution of this potential disparity will require a more detailed comparative analysis of CRP and TP0262 DNA-binding interactions. The authors candidly acknowledge the major limitation of their study: presently, methodology is not available to corroborate the results in *T. pallidum*. On the horizon, however, are technologies (e.g., chromatin immunoprecipitation) that promise to circumvent the refractoriness of spirochaetes to *in vitro* cultivation and genetic manipulation.

Clearly, our understanding of how *T. pallidum* manages its transcriptome is lagging far behind that of most other major bacterial pathogens. One of the most important outcomes of the present study is that it will help put to rest the pre-genomic view of the syphilis spirochaete as a transcriptionally invariant organism. For further evidence of the fierce and protracted duel between pathogen and host that ensues following inoculation, one need only look to the fact that *T. pallidum* encodes seven different sigma factors, as many as *E. coli*, and four more than the Lyme disease spirochaete, *Borrelia burgdorferi*, which must accommodate to both arthropod and mammalian milieus (Table 1). As Giacani and co-workers realize, TP0262 has a rich field in which to function and network. Both noteworthy and perplexing is that the *T. pallidum* repertoire consists of a mélange of *E. coli*- and *B. subtilis*-like sigma factors, including housekeeping (σ^A and σ^{70}) and stress-related sigma factors (σ^{24} and σ^B) from both lineages (Subramanian *et al.*, 2000; Fraser *et al.*, 1998; Fraser *et al.*, 1997; Hecker *et al.*, 2007; Raivio and Silhavy, 2001). *T. pallidum* also lacks RpoS, a general stress response regulator in *E. coli* (Hengge-Aronis, 2002) and a principal global regulator of differential gene expression during tick-to-mammal transmission of *B. burgdorferi* (Caimano *et al.*, 2007; Hubner *et al.*, 2001). Syphilologists are learning not to equate the biosynthetic limitations of *T. pallidum* with transcriptional and regulatory simplicity. From a pathogenesis standpoint, this emerging picture of a wily, adaptive adversary makes sense. The complex natural history of syphilis makes plain that the parasitic strategy of the spirochaete is ambitious. It is hard to imagine that the bacterium could implement such an agenda without invoking complex and versatile genetic programs.

References

- Busby S, Ebright RH. Transcription activation by catabolite activator protein (CAP). *J Mol Biol* 1999;293:199–213. [PubMed: 10550204]
- Caimano MJ, Iyer R, Eggers CH, Gonzalez C, Morton EA, Gilbert MA, et al. Analysis of the RpoS regulon in *Borrelia burgdorferi* in response to mammalian host signals provides insight into RpoS function during the enzootic cycle. *Mol Microbiol* 2007;65:1193–1217. [PubMed: 17645733]
- Centurion-Lara, A. Antigenic variation in *Treponema pallidum*. In: Radolf, JD.; Lukehart, SA., editors. *Pathogenic Treponema: Molecular and Cellular Biology*. Norwich, UK: Caister Academic Press; 2006. p. 267–284.
- Centurion-Lara A, Castro C, Barrett L, Cameron C, Mostowfi M, Van Voorhis WC, Lukehart SA. *Treponema pallidum* major sheath protein homologue TprK is a target of opsonic antibody and the protective immune response. *J Exp Med* 1999;189:647–656. [PubMed: 9989979]
- Cox, DL.; Radolf, JD. Metabolism of the *Treponema*. In: Radolf, JD.; Lukehart, SA., editors. *Pathogenic Treponema: Molecular and Cellular Biology*. Norwich, UK: Caister Academic Press; 2006. p. 61–100.
- Desrosiers DC, Sun YC, Zaidi AA, Eggers CH, Cox DL, Radolf JD. The general transition metal (Tro) and Zn(2+) (Znu) transporters in *Treponema pallidum*: analysis of metal specificities and expression profiles. *Mol Microbiol* 2007;65:137–152. [PubMed: 17581125]
- Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, et al. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 1997;390:580–586. [PubMed: 9403685]
- Fraser CM, Norris SJ, Weinstock GM, White O, Sutton GG, Dodson R, et al. Complete genome sequence of *Treponema pallidum*, the syphilis spirochaete. *Science* 1998;281:375–388. [PubMed: 9665876]

- Giacani L, Godornes C, Puray-Chavez M, Guerra-Giraldez C, Trompa M, Lukehart SA, Centurion-Lara A. TP0262 is a modulator of promoter activity of *tpr* subfamily II genes of *Treponema pallidum* subsp. *pallidum*. *Mol Microbiol*. 2009 in press.
- Giacani L, Hevner K, Centurion-Lara A. Gene organization and transcriptional analysis of the *tprJ*, *tprI*, *tprG*, and *tprF* loci in *Treponema pallidum* strains Nichols and Sea 81-4. *J Bacteriol* 2005;187:6084–6093. [PubMed: 16109950]
- Giacani L, Lukehart S, Centurion-Lara A. Length of guanosine homopolymeric repeats modulates promoter activity of subfamily II *tpr* genes of *Treponema pallidum* ssp. *pallidum*. *FEMS Immunol Med Microbiol* 2007;51:289–301. [PubMed: 17683506]
- Gonzalez CF, Stonestrom AJ, Lorca GL, Saier MH Jr. Biochemical characterization of phosphoryl transfer involving HPr of the phosphoenolpyruvate-dependent phosphotransferase system in *Treponema denticola*, an organism that lacks PTS permeases. *Biochem* 2005;44:598–608. [PubMed: 15641785]
- Gorke B, Stulke J. Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. *Nat Rev Microbiol* 2008;6:613–624. [PubMed: 18628769]
- Gosset G, Zhang Z, Nayyar S, Cuevas WA, Saier MH Jr. Transcriptome analysis of Crp-dependent catabolite control of gene expression in *Escherichia coli*. *J Bacteriol* 2004;186:3516–3524. [PubMed: 15150239]
- Hazlett KRO, Cox DL, Sikkink RA, Auch'ere F, Rusnak F, Radolf JD. Contribution of neelaredoxin to oxygen tolerance by *Treponema pallidum*. *Methods Enzymol* 2002;353:140–156. [PubMed: 12078490]
- Hecker M, Pane-Farre J, Volker U. σ^B -dependent general stress response in *Bacillus subtilis* and related Gram-positive bacteria. *Annu Rev Microbiol* 2007;61:236.
- Henge-Aronis R. Recent insights into the general stress response regulatory network in *Escherichia coli*. *J Mol Microbiol Biotechnol* 2002;4:341–346. [PubMed: 11931567]
- Hubner A, Yang X, Nolen DM, Popova TG, Cabello FC, Norgard MV. Expression of *Borrelia burgdorferi* OspC and DbpA is controlled by a RpoN-RpoS regulatory pathway. *Proc Natl Acad Sci U S A* 2001;98:12724–12729. [PubMed: 11675503]
- LaFond RE, Lukehart SA. Biological basis for syphilis. *Clin Microbiol Rev* 2006;19:29–49. [PubMed: 16418521]
- Lukehart, SA. Syphilis. In: Brauwald, E.; Fauci, AS.; Hauser, SL.; Longo, DL.; Jameson, JL., editors. *Harrison's Principles of Internal Medicine*. New York: McGraw Hill; 2004. p. 977-985.
- Norris SJ. Antigenic variation with a twist--the *Borrelia* story. *Mol Microbiol* 2006;60:1319–1322. [PubMed: 16796669]
- Norris, SJ.; Weinstock, GM. Comparative genomics of spirochaetes. In: Radolf, JD.; Lukehart, SA., editors. *Pathogenic Treponema: Molecular and Cellular Biology*. Norwich, U.K.: 2006. p. 19-38.
- Passner JM, Steitz TA. The structure of a CAP-DNA complex having two cAMP molecules bound to each monomer. *Proc Natl Acad Sci U S A* 1997;94:2843–2847. [PubMed: 9096308]
- Radolf, JD.; Hazlett, KRO.; Lukehart, SA. Pathogenesis of Syphilis. In: Radolf, JD.; Lukehart, SA., editors. *Pathogenic Treponemes: Cellular and Molecular Biology*. Norfolk, UK: Caister Academic Press; 2006. p. 197-236.
- Radolf JD. *Treponema pallidum* and the quest for outer membrane proteins. *Mol Microbiol* 1995;16:1067–1073. [PubMed: 8577243]
- Raivio TL, Silhavy TJ. Periplasmic stress and ECF sigma factors. *Annu Rev Microbiol* 2001;55:591–624. [PubMed: 11544368]
- Subramanian G, Koonin EV, Aravind L. Comparative genome analysis of the pathogenic spirochaetes *Borrelia burgdorferi* and *Treponema pallidum*. *Infect Immun* 2000;68:1633–1648. [PubMed: 10678983]
- Warner JB, Lolkema JS. CcpA-dependent carbon catabolite repression in bacteria. *Microbiol Mol Biol Rev* 2003;67:475–490. [PubMed: 14665673]
- Zheng D, Constantinidou C, Hobman JL, Minchin SD. Identification of the CRP regulon using *in vitro* and *in vivo* transcriptional profiling. *Nucleic Acids Res* 2004;32:5874–5893. [PubMed: 15520470]

Figure 1. Carbon catabolite repression (CCR) in *E. coli* and *B. subtilis*

In *E. coli*, the EIIA domain of the glucose transporter (EIIA^{Glc}) is the central processing unit in CCR. When phosphorylated, EIIA^{Glc} binds and activates adenylate cyclase (AC), which leads to cyclic AMP (cAMP) synthesis. In its non-phosphorylated form, EIIA^{Glc} cannot activate AC. In this case, EIIA^{Glc} binds and inactivates metabolic enzymes and transporters of secondary carbon sources, such as GlpK, LacY and other proteins (not shown). The phosphorylation state of EIIA^{Glc} is determined by phosphoenolpyruvate–carbohydrate phosphotransferase system (PTS) transport activity and the intracellular phosphoenolpyruvate (PEP) to pyruvate ratio, which decreases during high fluxes through glycolysis. In *B. subtilis*, histidine protein (HPr) can be phosphorylated at Ser46 by the HPr kinase/ phosphatase (HPrK). This phosphorylation occurs when the intracellular concentrations of fructose-1,6-bisphosphate (FBP) and ATP are high, which reflects the presence of preferred carbon sources. HPr(Ser-P) binds to the CcpA protein, and this interaction is enhanced by glycolytic intermediates, such as FBP and glucose-6-phosphate. The complex of CcpA and HPr(Ser-P) binds to *cre* sites on the DNA, and thereby represses the transcription of catabolic genes. In addition, HPr(His-P) contributes to CCR. In the absence of glucose, HPr(His-P) phosphorylates the glycerol kinase GlpK and transcriptional regulators that contain phosphoenolpyruvate–carbohydrate phosphotransferase system-regulatory domains (PRDs). Thus, in the presence of glucose, activation of the PRD regulators by their inducers is prevented. The dashed arrows show phosphate transfer; Glu-6-P, glucose-6-phosphate. Reprinted by permission from Macmillan Publishers LTD: Nature Reviews Microbiology 6:613–625; 2008 (<http://www.nature.com/nrmicro/journal/v6/n8/abs/nrmicro1932.html>).

Figure 2. *T. pallidum* encodes four proteins (TP0089, TP0261, TP0262, and TP0995) that contain cyclic nucleotide monophosphate (cNMP) binding domains, one of which (TP0262) is a close structural orthologue of the *E. coli* CRP

(A) Amino acid sequence alignment with the *E. coli* CRP. For those homologues other than TP0262, only the cNMP binding domains are shown; TP0089 has two, which are aligned separately. Identical and similar amino acids are outlined and shaded in dark and light grey, respectively. Blue, red, and blue/red circles demarcate the residues of the *E. coli* Crp that bind to cAMP, DNA, and both molecules, respectively. (B) X-ray crystal structure of the *E. coli* CRP (red) bound to cAMP (yellow) and DNA (light grey). Shown in blue is the homology model of Tp0262 aligned to the *E. coli* protein. PDB code: 2CGP. The structural model for TP0262 is based on Passner and Steitz (1997).

Sigma factors in *T. pallidum*

Table 1

Sigma Factor	Function	<i>E. coli</i>	<i>B. subtilis</i> ^a	<i>T. pallidum</i>	<i>B. burgdorferi</i>
σ^{70} (RpoD)	General transcription	✓		✓	✓
σ^{54} (RpoN)	Nitrogen metabolism	✓		✓	✓
σ^{28} (RpoF)	Motility	✓		✓	
σ^{32} (RpoH)	Heat shock response	✓			
σ^{24} (RpoE)	Extracytoplasmic function	✓		✓	
σ^{38} (RpoS)	General stress	✓			✓
σ^{19} (FecI)	Fe(III)-citrate transport	✓			
σ^A (SigA, RpoD)	General transcription		✓	✓	
σ^B (SigB, RpoS)	General Stress		✓	✓	
σ^G (SigG)	Sporulation		✓	✓	

^aOnly those sigma factors for which *T. pallidum* has orthologues are shown.