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Molecular Signaling Mechanisms of the Periopathogen, *Treponema denticola*

ABSTRACT

In the healthy subgingiva, oral treponemes account for a small percentage of the total bacteria. However, in diseased periodontal pockets, treponemes thrive and become a dominant component of the bacterial population. Oral treponemes are uniquely adept at capitalizing on the environmental conditions that develop with periodontal disease. The molecular basis of adaptive responses of oral treponemes is just beginning to be investigated and defined. The completion of several treponeme genome sequences and the characterization of global regulatory systems provide an important starting point in the analysis of signaling and adaptive responses. In this review, we discuss existing literature focused on the genetic regulatory mechanisms of *Treponema denticola* and present an overview of the possible roles of regulatory proteins identified through genome analyses. This information provides insight into the possible molecular mechanisms utilized by oral spirochetes to survive in the periodontal pocket and transition from a minor to a dominant organism.

KEY WORDS: treponemes, two-component regulatory systems, AtcRS, Hpk2, Rrp2, c-di-GMP.

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INTRODUCTION

Periodontal disease, a common health problem of middle-aged adults, affects millions every year, and its costs to society are high (Dye *et al*., 2007). Periodontitis has been aptly referred to as a "polymicrobial disruption of host homeostasis," the intensity of which is determined by variables including the composition of the oral flora, host genetic predisposition, and underlying disorders (Darveau, 2010). The bacteriology of periodontal disease is complex, due to the presence of nearly 700 bacterial species in the oral cavity, with ~ 400 found in association with subgingival plaque (Paster *et al*., 2006). As periodontal disease develops, a shift occurs in the relative abundance of specific species. Elevated numbers of *Porphyromonas gingivalis, Tannerella forsythia*, and *Treponema denticola*, which form the red-microbial complex, correlate with gingivitis, chronic periodontitis, acute necrotizing ulcerative gingivitis, endodontic lesions, and have been associated with cardiovascular disease, stroke, endometriosis, preterm delivery of low-birthweight infants, and diabetes mellitus (Socransky *et al*., 1998; Yuan *et al*., 2001; Kshirsagar *et al*., 2007; Chen *et al*., 2008; Makiura *et al*., 2008; Kavoussi *et al*., 2009; Inaba and Amano, 2010). While oral *Treponemes* constitute a low percentage of the bacterial population in gingival crevicular fluid of healthy individuals, they are abundant in periodontal pockets (Dewhirst *et al*., 2000; Ellen and Galimanas, 2005). Due to *T. denticola's* abundance at diseased sites, its close association with other periopathogens (*Porphyromonas gingivalis*), its localization at the plaque-tissue interface, and its production of powerful proteases, it is considered a prime contributor to periodontal-disease-associated tissue destruction (Holt and Ebersole, 2005). *T. denticola*, which produces dentilisin (a chymotrypsin-like protease) (Fenno *et al*., 1998), disrupts epithelial cells *in vitro* in a dentilisin-dependent manner (Chi *et al*., 2003). A direct role in tissue destruction *in vivo* has not been demonstrated. However, *T. denticola* does reside within epithelial cells in individuals with chronic periodontitis (Colombo *et al*., 2007). Periodontal-disease-associated tissue destruction leads to drastic changes in the physiochemical environment of the subgingival crevice and periodontium. This review seeks to summarize recent progress that has been made in delineating the key regulatory proteins and molecules that mediate environmental sensing and adaptive responses of *T. denticola*, and to provide potential insight into the molecular mechanisms that allow this periopathogen to thrive in disease sites in the periodontium.

SPIROCHETES

Spirochetes are a diverse group of bacteria that share a common spiral-shaped or flat-wave-form ultrastructure. The phylum *Spirochaetes* branched early from the evolutionary tree, dividing into 3 families (*Spirochaetaceae, Brachyspiraceae*, and *Leptospiraceae*) and 9 genera. Of these, the *Treponema, Borrelia*, and *Leptospira* are human health concerns. The genus *Treponema* includes the causative agents of syphilis and periopathogens. The *Leptospira*

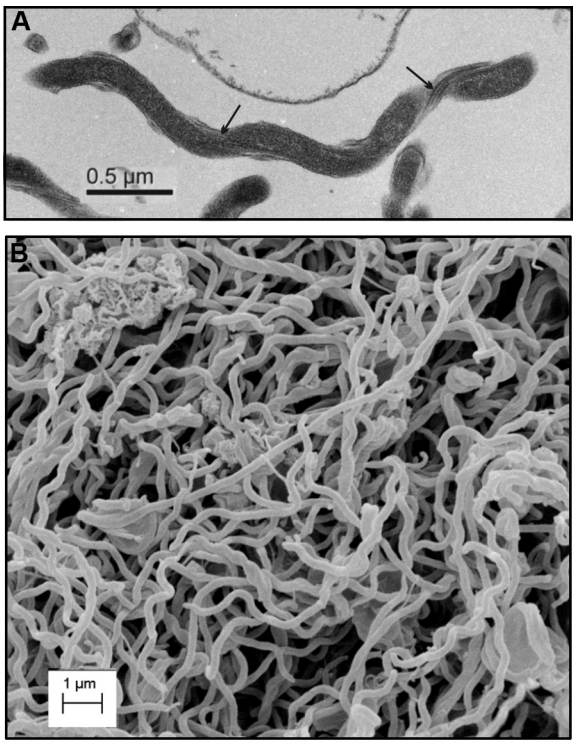

Figure 1. Electron microscopic analysis of *T. denticola*. Transmission **(A)** and scanning **(B)** electron micrographs of *T. denticola* ATCC 35405. Scale bars are indicated. The arrows in panel A indicate the endoflagella bundles.

are causative agents of leptospirosis, and the *Borrelia* cause relapsing fever and Lyme disease. Spirochetes possess a distinctive ultrastructure (Fig. 1) and a unique mode of motility. They range from 5 to 20 µm in length and 0.1 to 0.5 µm in diameter. They are similar to Gram-negative bacteria, possessing both inner and outer membranes (Fig. 2), but most species lack lipopolysaccharide (LPS). LPS is replaced by lipoproteins and glycolipids (Radolf and Lukehart, 2006; Samuels and Radolf, 2010).

Spirochete ultrastructure is defined by the physical influence of internal flagella (endoflagella). Endoflagella form two distinct bundles that are contained within the periplasmic space, with each inserting into the inner membrane at opposite ends of the cell [reviewed in Charon *et al*. (1992)]. The bundles extend two-thirds the length of the cell, wrapping around the inner membrane in a right-handed sense (refer to Fig. 1A). Intriguing questions remain as to how spirochetes regulate directionality of movement and chemotactic responses. *Treponema denticola* devotes nearly 6% of its genome to genes involved in motility and chemotaxis. The unusually high number of methyl-accepting chemotaxis proteins implies that it can respond to a wide range of chemoattractants (Seshadri *et al*., 2004). Known chemoattractants include serum, albumin, and glucose (Ruby *et al*., 2008). Much remains to be determined regarding the regulatory mechanisms of spirochetal motility and chemotaxis (Radolf and Lukehart, 2006; Samuels and Radolf, 2010). Recent studies indicate that the secondary messenger molecule, c-di-GMP, may be a key regulator of chemotaxis and motility in other spirochetes (Rogers *et al*., 2009; Sultan *et al*., 2010). The possible role of c-di-GMP in treponemal biology is discussed in detail below.

PHYLOGENETICS AND GENOME COMPOSITION OF THE ORAL TREPONEMES

The human oral microbiome harbors 76 treponemal phylotypes, with *T. denticola* being the most prevalent (Paster *et al*., 1991). *T. denticola* strain ATCC 35405, the most extensively characterized oral treponeme, possesses a 2.84-Mb circular chromosome (2786 ORFs) (Seshadri *et al*., 2004). *T. vincentii* ATCC 35580 and *T. lecithinolyticum* OMZ684T harbor 2.51-Mb (2559 ORFs) and 1.47-Mb (2059 ORFs) chromosomes, respectively (www.homd.com). Approximately 1200 of *T. denticola's* ORFs have assigned functions. Consistent with their fastidious growth requirements, the genomes of oral treponemes carry only a limited set of genes that are associated with biosynthetic pathways (Seshadri *et al*., 2004). Plasmids are not a significant genomic component of oral treponemes (Chan *et al*., 1996).

TWO-COMPONENT REGULATORY (TCR) SYSTEMS

The mechanisms used by oral treponemes to sense and respond to the physiochemical changes associated with periodontal disease remain largely unknown. Microarray studies have demonstrated that the *T. denticola* transcriptome is responsive to heat shock, oxygen shock, and osmotic downshift (McHardy *et al*., 2010). In bacteria, two-component regulatory (TCR) systems sense and transmit signals in response to environmental stimuli (Galperin, 2006). TCR systems typically consist of a histidine kinase and a response regulator that can vary in domain architecture and output effector mechanisms (Galperin, 2006). Typically, signal transduction is initiated with the sensing of stimuli by histidine kinases. Histidine kinases autophosphorylate and then transfer the phosphate to the receiver domain of a response regulator protein, inducing conformational changes that activate the protein. In some hybrid kinases, which possess an integrated response regulator receiver domain, the phosphate may undergo intramolecular transfer steps before being transferred to its final aspartate residue. Hybrid kinases may use accessory proteins to complete the phosphotransfer process. The mechanistic and domain architecture diversity of TCR systems has been detailed in several reviews (Galperin *et al*., 2001; Galperin, 2006). Many response regulators act at the transcriptional level. However, some lack DNA-binding domains and regulate through protein-protein interactions or the production of secondary messenger molecules.

Most bacteria encode 20 to 30 histidine-kinase response-regulator pairs. This allows for the fine-tuned control of responses to environmental stimuli (Galperin *et al*., 2001). *T. denticola* strain 35405 encodes 6 histidine kinases, 7 response regulators, and 2 kinase-response regulator hybrids that have potential global regulatory ability (Table 1). *T. lecithinolyticum* and *T. vincentii* encode considerably fewer histidine kinases and response regulators. *T. denticola* TCR systems may offer a

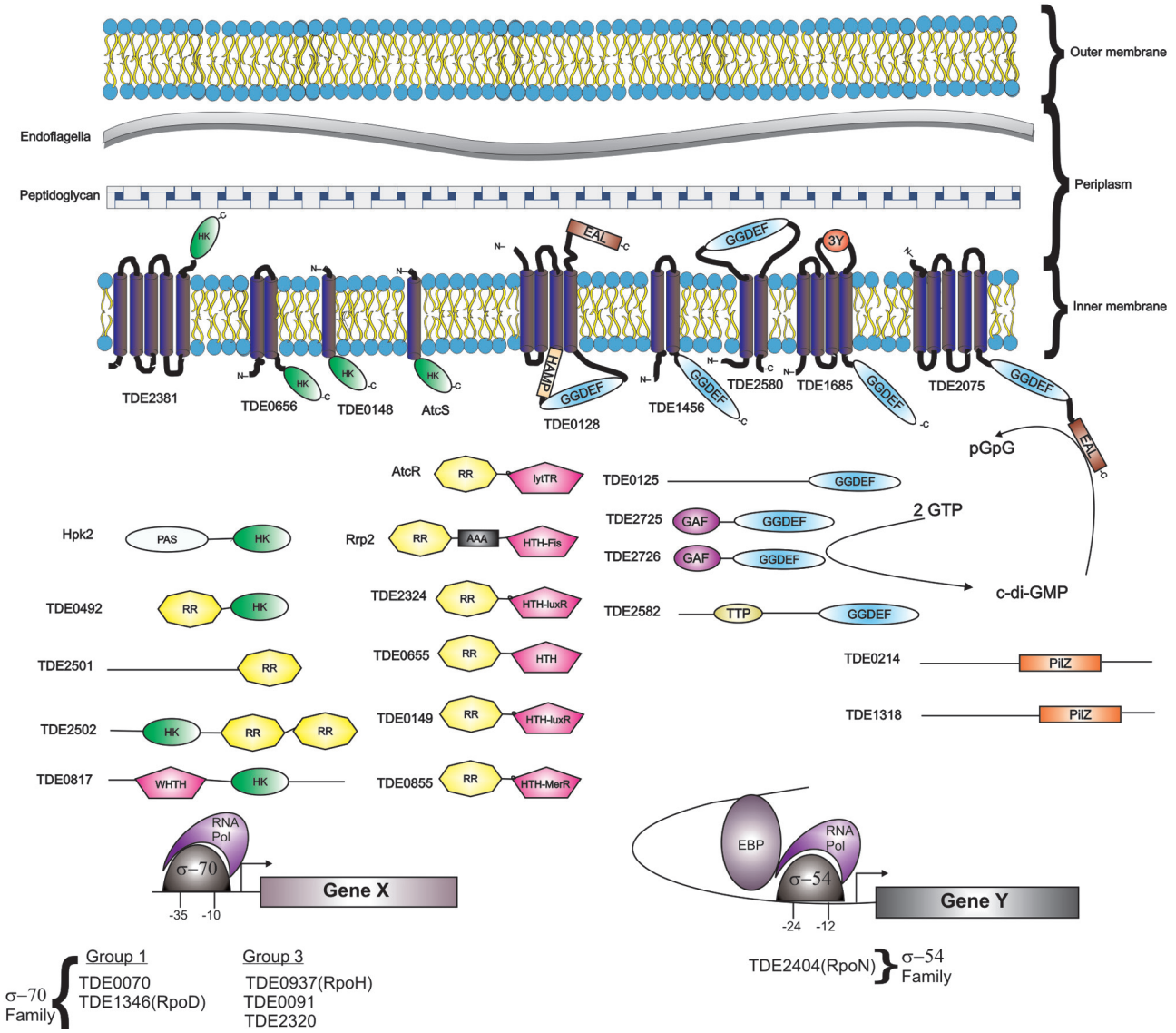

Figure 2. Cellular architecture and genetic regulatory and signaling networks of *T. denticola* 35405. The *T. denticola* cellular architecture and predicted location of regulatory and signaling proteins are depicted. The membrane topology of the N- and C-termini of each protein is indicated. The functional domains of specific ORFs are indicated by color coding and abbreviations as follows: histidine kinase (HK-green), response regulator receiver (RR, yellow), GGDEF (blue), EAL (brown), and PilZ (orange). DNA-binding domains, which are color-coded pink, are labeled with their subfamily designation (HTH, helix-turn-helix; WHTH, winged helix-turn-helix; lytTR/AlgR/AgrA/LytR family). Other functional domains indicated include TCR 3Y motif (labeled as 3Y), tetratricopeptide repeat domain (TTP), HAMP dimerization domain (HAMP), AAA ATPase domain (AAA), GAF, and PAS domains. The sigma factors of *T. denticola* are listed at the bottom, with the schematic indicating the general way in which they interact with DNA and/or other regulatory proteins. Enhancer binding proteins are abbreviated as EBP.

biological advantage to this periopathogen. Determination of additional genome sequences will provide insight into the potential adaptive capabilities of other treponemes. With information derived from the *T. denticola* strain 35405 genome sequence (Seshadri *et al*., 2004), a schematic depicting putative regulatory mechanisms and networks was generated (Fig. 2). This model is speculative. It is clear that our understanding of signaling mechanisms in oral treponemes as a whole is in its infancy and requires considerable investigation.

Two *T. denticola* strain 35405 TCR systems have been identified that appear to have potential global regulatory capability: AtcRS (Frederick *et al*., 2008) and Hpk2-Rrp2 (Sarkar *et al*., 2010) (Fig. 3). The AtcS sensor kinase and AtcR response regulator are encoded by TDE0032 and TDE0033, respectively. The Hpk2 sensor kinase and Rrp2 response regulator are encoded by TDE1970 and TDE1969, respectively. These genes are highly conserved among *T. denticola* isolates, and the putative functional residues are invariant in sequence. Interestingly, orthologs of AtcR and AtcS are not found in other spirochetes for which genome sequences are known. The AtcRS regulatory network may, in part, define the unique biological properties of *T. denticola*. In contrast, the Hpk2-Rrp2 system may have a

Table 1. Histidine Kinases and Response Regulators of *T. denticola* Strain 35405

ORF	Notes
Histidine Kinases	
TDE0032(AtcS)	forms a TCR system with the response regulator, AtcR; transcription is up-regulated during late-stage growth
TDE0148	forms a TCR system with TDE0149; single transmembrane (TM) domain; co-transcribed withTDE149 and TDE0150 (a ribonuclease containing a cyclic- nucleotide-binding domain).
TDE0656	forms a TCR system with TDE0655; 2 TM domains; may function as an intramembrane sensor; may be transcribed as part of an operon consisting of TDE0658-TDE0653; analogous locus is conserved in *Bacillus*
TDE1970(Hpk2)	no TM domain; N-terminal PAS domain; significant homology with Hpk2 of *B. burgdorferi*
TDE0817	orphan kinase; no TM domain; N-terminal winged helix-turn-helix DNA-binding domain
TDE2381	orphan kinase with a periplasmic kinase domain; 5 TM domains; adjacent to genes involved in cobalamine metabolism (TDE2382 and TDE2383).
Response Regulators	
TDE0033(AtcR)	forms a TCR system with AtcS; only spirochetal protein with a LytTR DNA-binding domain
TDE0149	LuxR helix-turn-helix domain
TDE0655	helix-turn-helix domain
TDE1969(Rrp2)	RpoN interaction domain and a Fis DNA-binding domain
TDE2501	forms a TCR system with TDE2502; lacks an obvious effector domain
TDE0855	orphan response regulator; excisionase DNA-binding domain; homologous protein (74% similar) found in *Spirochaeta*; may be co-transcribed with TDE0856-TDE0859
TDE2324	orphan response regulator; LuxR helix-turn-helix domain; located upstream of an eight-TM domain containing hypothetical protein (TDE2325) and a cobalamine synthesis protein cobQ (TDE2326)
Hybrids	
TDE0492	homology with a TCR system of *S. aureus* that is involved in virulence and biofilm formation; no TM domain
TDE2502	consists of a kinase domain and 2 receiver domains; possibly co- transcribed with TDE2501; no TM domain

more universal function, since it is present in several other spirochete species, including the *Borrelia*.

AtcS is a 29-kDa inner membrane anchored protein with the characteristic H (his phosphorylation domain), N and G boxes (nucleotide-binding domains) of histidine kinases. The H box harbors 3 possible autophosphorylation sites (H57, H60, and H62). H57 and H60 are predicted to project into a solvent-accessible, putative nucleotide-binding pocket and be properly positioned to accept phosphate (Frederick *et al*., 2008). AtcS has 43 to 65% amino acid similarity with the CitA domain of the C4 dicarboxylate sensor kinases of *Bacillus* (Janausch *et al*., 2002). The highest degree of homology occurs within the kinase domains. The kinase domain interacts with its cognate response regulator, thus allowing for intermolecular phosphotransfer (Ohta and Newton, 2003). Hence, the homology between AtcS and CitA most likely reflects the structural conservation that is required for the kinase response-regulator interaction. The mechanism by which AtcS senses environmental signals, and the nature of those signals, remains to be determined, since the protein lacks an obvious sensing domain.

AtcR is predicted to be a 28-kDa cytoplasmic protein with 3 possible phosphor-accepting sites (D52, D54, and D59) (Frederick *et al*., 2008). AtcR displays 60% amino acid similarity with the *Clostridium* VirR response regulator (Ba-Thein *et al*., 1996). Both VirR and AtcR possess a LytTR domain. LytTR domains were originally identified in the AlgR/AgrA/LytR family of transcriptional regulators (Nikolskaya and Galperin, 2002). LytTR binding motifs have been mapped upstream of environmentally regulated genes, suggesting a role for AtcR in adaptive responses. Of the nearly 6000 sequenced response regulator proteins, only 5% harbor LytTR domains. The *T. denticola* AtcR protein is the only known oral spirochete protein to harbor a LytTR domain. The completion of additional treponemal genome sequences will further our understanding of the distribution of this regulatory domain and its role in oral spirochete biology.

Hpk2 is a conserved 46-kDa protein that is universally distributed among *T. denticola* strains (Sarkar *et al*., 2010). While *T. vincentii* encodes an Hpk2 ortholog (56% amino acid identity and 75% similarity), there is no obvious ortholog in *T. lecithinolyticum*. Hpk2 harbors an N-terminal PAS domain (acronym for Per-ARNT-Sim). PAS domains detect specific environmental stimuli, including oxygen (Moglich *et al*., 2009). It is noteworthy that the *T. denticola* Hpk2 PAS domain harbors a unique 15-aa insertion that is not found in any other PAS-domain-containing protein. This unique insertion may modulate Hpk2 signaling activity. The PAS domain is followed by an H-Box with 3 possible autophosphorylation sites (H185, H197, and H219) and an H-ATPase domain (ATP-Mg^{2+}-binding sites) that spans the C-terminal third of the protein.

T. denticola Rrp2 is a conserved 52-kDa protein carried by several spirochete species, including the *Borrelia* (Sarkar *et al*., 2010). *T. vincentii* (68% amino acid identity and 84% similarity), but not *T. lecithinolyticum*, harbors an Rrp2 ortholog. Rrp2 possesses 3 putative phosphor-acceptor residues (D45, D48, and D53) in its receiver domain. The *B. burgdorferi* Rrp2 ortholog is a σ^{54}-dependent response regulator that is an essential activator of the RpoN-RpoS regulatory pathway (Yang *et al*., 2003). RpoN up-regulates RpoS, which positively regulates genes involved in the transmission cycle (Ouyang *et al*., 2008). In *B. burgdorferi*, replacement of wild-type *rrp2* with a site-directed mutant deficient in ATP binding abolished the transcriptional expression of several important, plasmid-encoded virulence factors that are also regulated by temperature and pH (Revel *et al*., 2002; Ojaimi *et al*., 2003). As in the *Borrelia, T. denticola* Rrp2

may regulate transcriptional responses to environmental changes. Interestingly, *T. denticola* lacks RpoS (Seshadri *et al*., 2004); hence, the mechanisms of Rrp2-mediated gene regulation may be distinctly different from those of other spirochetes.

The ability of AtcS and Hpk2 to autophosphorylate and transfer phosphate to their cognate response regulators has been demonstrated with recombinant proteins (Frederick *et al*., 2008; Sarkar *et al*., 2010). Neither TCR system uses accessory proteins, since transfer occurs with defined purified proteins. Interestingly, for both systems, phosphotransfer requires pre-loading of the kinase prior to incubation with the response regulator. Phosphotransfer requires kinase dimerization to generate a stable structure that can interact with the response regulator (Ohta and Newton, 2003). If the kinase is not preloaded with phosphate, unphosphorylated response regulator may interact with kinase monomers, preventing dimerization and inhibiting interactions required for phosphotransfer.

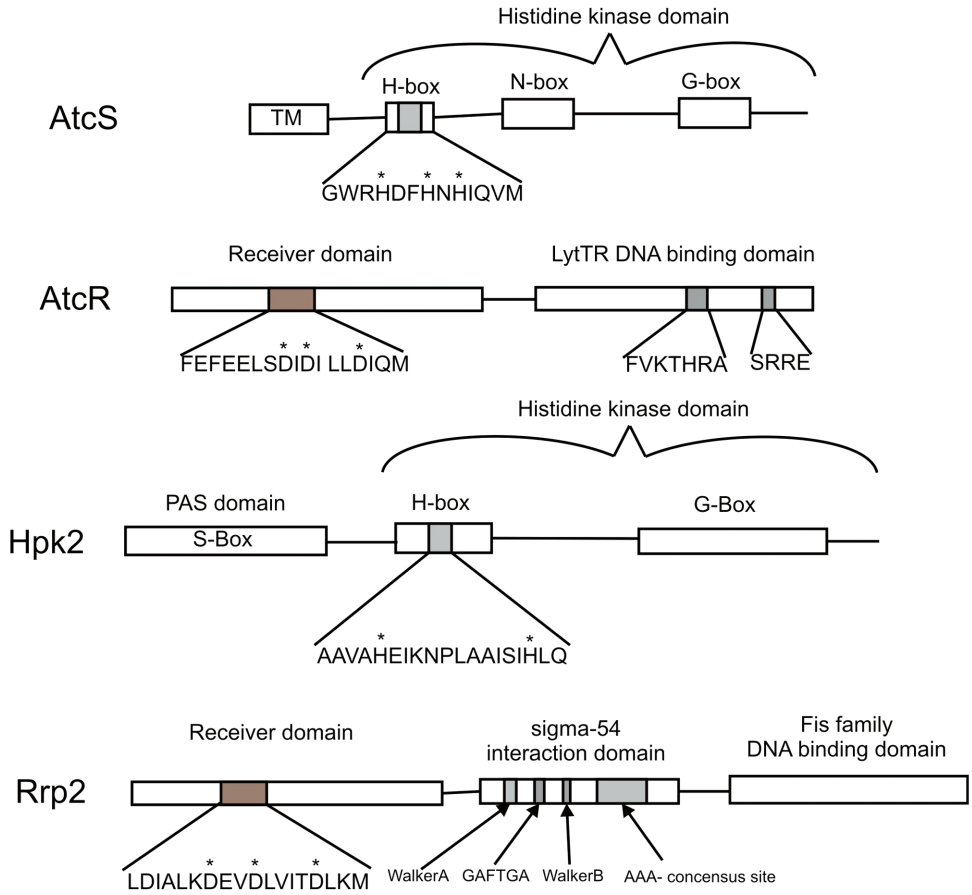

Figure 3. Domain architecture of the AtcSR and Hpk2-Rrp2 systems. The putative functional domains of each protein are indicated. The specific sequences of regions that undergo phosphorylation are indicated, with the likely target residues designated by an asterisk. In addition, the residues required for LytTR functional activity are also shown.

The PAS Domain of Hpk2 Influences Responsiveness to Environmental Stimuli

T. denticola is an obligate anaerobe, and, upon exposure to micro-aerophilic conditions, it produces hydrogen sulfide which serves to deplete localized oxygen, thus restoring an anaerobic microenvironment (Lai and Chu, 2008). The presence of a heme-binding pocket in the Hpk2 PAS domain suggests that this protein could play a role in sensing oxygen. In light of the significant differences in oxygen concentration that exist in specific microenvironments within periodontal pockets (Mettraux *et al*., 1984), the ability to respond to even small concentration changes could be a key aspect of *T. denticola* biology. Sarkar and co-workers tested the kinase activity of an N-terminal truncation variant lacking the PAS domain (Hpk2ΔPAS) (Sarkar *et al*., 2010). No significant difference in phosphate incorporation was observed for full-length Hpk2 and Hpk2ΔPAS ($p > 0.05$) when the assays were conducted under aerobic conditions. However, under anaerobic conditions, a significant reduction in phosphate incorporation was observed with Hpk2ΔPAS (relative to Hpk2; $p < 0.05$). Hpk2ΔPAS was also unable to transfer phosphate to Rrp2. Analysis of the data suggests that, under anaerobic conditions, signaling through the PAS domain is required for optimal autophosphorylation activity.

Transcriptional Analyses of *atcR, atcS, hpk2*, and *rrp2*

Consistent with that observed for most histidine-kinase response-regulator cognate pairs, *atcR* and *atcS* and *hpk2* and *rrp2* are co-transcribed (Frederick *et al*., 2008; Sarkar *et al*., 2010). Both operons are up-regulated during late-log-phase growth and can be expressed, at least in part, as larger operons (Frederick *et al*., 2008; Sarkar *et al*., 2010). *AtcR-atcS* is transcribed as an operon consisting of ORFs TDE0037(*abrB)*-TDE0030 and as a smaller bicistronic transcript. *Hpk2-rrp2* is transcribed as an operon consisting of ORFs TDE1974-TDE1968. It remains to be determined if these large operons are the dominant transcriptional units during growth in the human host. Transcriptional start site analyses have revealed that the 2 *atcS-atcR* promoters are group 1 σ^{70} consensus-type promoters (Wosten, 1998; Frederick *et al*., 2008). The possible roles of alternative sigma factors in *T. denticola* biology are discussed below.

ORF TDE0037 (designated as AbrB), which can be co-transcribed with *atcR*, encodes a transition-state transcriptional regulatory protein (Frederick *et al*., 2008). AbrB family member proteins orchestrate gene expression patterns in response to environmental signals (Phillips and Strauch, 2002). Transcription of *abrB* is typically autoregulated and up-regulated during periods of growth transition. Analysis of the sequence upstream

from *T. denticola abrB* identified a consensus AbrB-binding site, suggesting that AbrB may be a key player in the growth-phase-dependent transcriptional patterns of *atcRS* (Frederick *et al*., 2008).

Consideration of the putative functions of proteins encoded by genes co-transcribed with *hpk2-rrp2* could provide insight as to how the up-regulation of this operon could contribute to the success of *T. denticola* in the periodontal pocket. TDE1968 (FtsJ), TDE1971 (DnaX), and TDE1974 (MurG) all encode proteins that carry out functions required for rapid growth. FtsJ, a 23S rRNA methyltransferase, stabilizes the 50S subunit of the 70S ribosome, allowing for efficient translation (Hager *et al*., 2002). In *B. burgdorferi*, inactivation of *ftsJ* results in impaired growth and morphological abnormalities (Morozova *et al*., 2005). DnaX (TDE1971) encodes the gamma/tau subunit of DNA polymerase III, a protein involved in DNA replication (Maki and Kornberg, 1988). MurG (TDE1974), a glycosyltransferase, catalyzes a terminal step in peptidoglycan synthesis. MurG is essential for cell viability and may be part of the divisome (Mohammadi *et al*., 2007). Last, ORFs TDE1972 and TDE1973 are annotated as a possible peptide toxin and colicin V production factor, respectively. While the specific role or activity of these particular proteins has not been directly demonstrated, it is possible that their production could inhibit the growth of competing organisms. The collective up-regulation of Hpk2-Rrp2, its adjacent genes, and other genes of the regulon could facilitate the rapid growth of *T. denticola* in the periodontal pocket.

OTHER REGULATORY NETWORKS IDENTIFIED THROUGH GENOME SEQUENCE DETERMINATION

C-di-GMP and Treponemal Biology

C-di-GMP, an important secondary-messenger molecule in bacteria, regulates cellular processes, including motility, expression of virulence genes, cell-to-cell signaling, exopolysaccharide production, and the transition between biofilm and planktonic modes of growth (Hengge, 2009). C-di-GMP is synthesized by diguanylate cyclases, which are defined by the presence of GGDEF domains (formerly referred to as the DUF1 domain). The intracellular pool of c-di-GMP, which can be highly localized within a cell, is controlled by the opposing activities of diguanylate cyclases and phosphodiesterases that possess an EAL or HD-GYP domain. GGDEF domains exist in several different contexts and have been identified in response-regulatory proteins (*B. burgdorferi* Rrp1) (Ryjenkov *et al*., 2005), in hybrid proteins that also contain an EAL domain, or as stand-alone enzymes (Romling *et al*., 2005). The effector mechanisms of c-di-GMP are just beginning to be defined. C-di-GMP has been shown to bind to PilZ domain-containing proteins, inducing conformational changes that modulate protein activity (Ryjenkov *et al*., 2006; Freedman *et al*., 2009). C-di-GMP has also been shown to bind to proteins that lack obvious PilZ domains (Chin *et al*., 2010). The determinants required for these non-PilZ-based interactions are not known. C-di-GMP also regulates at the mRNA level by binding to riboswitches (non-coding segments of mRNA that form specific secondary structures) (Sudarsan *et al*., 2008). The inability to identify conserved riboswitches in spirochetes could be reflective of sequence divergence consistent with the evolutionary separation of spirochetes from other organisms.

The importance of c-di-GMP in spirochetal biology is highlighted by recent studies of *B. burgdorferi* (Ryjenkov *et al*., 2005; Rogers *et al*., 2009; Freedman *et al*., 2009; Sultan *et al*., 2010). *B. burgdorferi* encodes a single diguanylate cyclase designated as response regulatory protein 1 (Rrp1). Microarray analyses of a *B. burgdorferi rrp1* deletion mutant revealed that Rrp1, and by extension c-di-GMP, regulates the transcription of nearly 10% of the genome (Rogers *et al*., 2009). The genes regulated encode proteins with diverse functions. Genome sequence analyses suggest that c-di-GMP may also serve as an important regulatory molecule in *T. denticola*. Strain 35405 harbors proteins with GGDEF, EAL, and PilZ domains (Table 2). One of the PilZ domain proteins (TDE0214) has significant homology to the PlzA protein of *B. burgdorferi* (Freedman *et al*., 2009). C-di-GMP has the potential to regulate numerous processes that are central to the ability of *T. denticola* to survive and thrive in the subgingival crevice. The analysis of the role of c-di-GMP and of proteins that constitute the c-di-GMP regulatory network in the biology of oral treponemes represents a fertile area for future research.

Sigma (σ) Factors and Anti-σ Factors of *T. denticola* 35405

The ability of bacteria to selectively regulate specific genes is dependent in part on the activity of sigma factors (σ). Sigma factors interact with RNA polymerase and direct binding to specific promoter sequences [reviewed in Wosten (1998)]. There are two main families of σ factors: σ^{70} and σ^{54}. The σ^{70} family proteins bind to consensus -10 and -35 regions upstream of the transcriptional start site. The σ^{54} proteins bind to conserved -12 and -24 regions. The σ^{70} family is further divided into 3 main groups that bind to different nucleotide sequences. All eubacteria produce one or more σ^{70} family member proteins. Group 1 σ^{70} proteins direct the transcription of genes required for exponential growth. Group 2 σ^{70} proteins recognize DNA sequences similar to those recognized by group 1, but these sigma factors are not essential for exponential growth or survival. The stationary phase σ factor, RpoS (σ^{38} or σ^{s}), is an example of a σ^{70} group 2 sigma factor. The σ^{70} group 3 proteins consist of the alternative σ factors that control the expression of regulons in response to specific environmental stimuli or during a tightly defined window during a life/developmental/pathogenesis stage. The flagellar σ factor, σ^{28}, extracytoplasmic function (ECF) σ factors, and heat-shock sigma factors are members of this group. *T. denticola* 35405 encodes 2 group 1 σ^{70} factors and 4 group 3 σ^{70} proteins (Table 3).

The activity of σ^{70} family sigma factors can be regulated by anti-sigma factors, anti-anti-sigma factors, and σ factor regulatory proteins. Two examples of such regulation are the FliA-FlgM system (Aldridge and Hughes, 2002) and the RsbUVW system originally described in Gram-positive bacteria

Table 2. C-di-GMP Regulatory Network Proteins of *T. denticola* 35405

Family	ORF	Notes
GGDEF	TDE0125	putative N-terminal ligand-binding domain
	TDE1456	terminal gene of a 5-gene operon (other genes are conserved hypotheticals); analogous locus is conserved in *T. vincentii*.
	TDE1685	3Y motif (occur in other sensor proteins)
	TDE2580	possible operon with TDE2581 and TDE2582
	TDE2581	homologous to GGDEF proteins but does not have a GGDEF domain.
	TDE2582	tetratricopeptide repeat domain (possible adhesion function)
	TDE2725	cyclic-nucleotide-binding domain; may be co-transcribed with TDE2726
	TDE2726	cyclic-nucleotide-binding domain; possibly arose through gene duplication of 2725; *T. vincentii* contains 2 homologs in tandem but lacks TDE2725
GGDEF/EAL	TDE0128	GGDEF and EAL domains are separated by a putative TM domain; HAMP domain
	TDE2075	possible operon with TDE2076 (solute binding protein).
PilZ	TDE0214	homolog of *B. burgdorferi* PlzA (c-di-GMP binding protein); possible operon with a tetratricopeptide repeat (TPR) protein
	TDE1318	only identifiable homolog is in *Spirochaeta thermophila* (34% identical); may form a 4-gene operon that includes genes involved in competence and recombination

Table 3. Sigma, Anti-sigma, and Anti-anti-sigma Factors of *T. denticola* 35405

Family/ Group	ORF	Notes and Comments
σ^{70} family		
Group 1	TDE0070	lacks N-terminal domain required for open complex formation which could render the protein non-functional
	TDE1346 (RpoD)	regulates housekeeping genes; conserved among spirochetes
Group 2	None detected	
Group 3	TDE0937 (RpoH)	heat-shock-response sigma factor
	TDE0091	ECF family; part of operon containing the anti-σ factor TDE0090, cysteinyl-tRNA synthetase (CysS), and a transglutaminase; suggests possible role in cysteine and glutathione metabolism
	TDE2320	ECF family; putative operon with TDE2319 and TDE2321 (hypotheticals)
	TDE2683 (FliA)	σ^{28}; regulator of flagellar biosynthesis
σ^{54} family	TDE2404 (RpoN)	Possible regulator of global transcriptional responses to environmental stimuli
Anti-σ factors	TDE00201 (FlgM)	Possible regulator of σ^{28}
	TDE1121	RsbW homolog; likely co-transcribed with its cognate anti-anti-σ factor (TDE1122) and the σ factor regulatory protein (TDE1123).
	TDE0090	Possible regulator of TDE0091 (see above)
	TDE2319	Possible regulator of TDE2320 (see above)
Anti-anti-σ factors	TDE0632	RsbV homolog; putative operon with TDE0630 and TDE0631
	TDE1122	RsbV homolog; putative operon with TDE1123
	TDE2083	Contains a zinc finger DNA-binding domain.
σ factor regulatory protein	TDE0630	RsbU homolog; 2 TM domains with a cytoplasmic phosphatase domain; may regulate TDE0632
	TDE0631	RsbU homolog; 10 TM domains with a periplasmic phosphatase domain; may regulate TDE0632
	TDE1123	RsbU homolog: 3 TM domains, a GAF domain and phosphatase domain; may regulate TDE1122

(Pane-Farre *et al*., 2009). Homologs of all of the key players of these systems are encoded by the *T. denticola* 35405 genome (Table 3), and would contribute to the signaling capacity of the organism.

Members of the σ^{54} family of σ factors are structurally distinct from the σ^{70} proteins and regulate by a different mechanism. They require a separate transcription factor, the enhancer binding protein (EBP), to direct transcription (Buck *et al*., 2000). The sole σ^{54} protein encoded by *T. denticola* is RpoN (TDE2404). The *T. denticola* response regulator, Rrp2, which is discussed in detail above, possesses a σ^{54} (*i.e*., RpoN) interaction domain (Sarkar *et al*., 2010). Rrp2 most likely serves as an EBP for RpoN. In *Borrelia burgdorferi*, the Rrp2-RpoN-RpoS system controls the transcription of several environmentally regulated virulence factors (Hubner *et al*., 2001; Smith *et al*., 2007). However, the lack of an RpoS (σ^{38}) homolog in *T. denticola* suggests that RpoN may either act directly or utilize a yet-to-be-identified regulatory partner. *T. denticola* encodes at least 3 proteins that contain σ^{54} interaction domains (TDE2079, TDE2309, and TDE2593). TDE2309 and TDE2079 contain GAF domains which are associated with the binding of cyclic nucleotides and small molecules (Martinez *et al*., 2002).

Interestingly, homologs of these σ^{54} interaction domain-containing proteins are not found in *T. vincentii* or *T. lecithinolyticum*.

CONCLUSIONS AND FUTURE DIRECTIONS

As periodontal disease develops, dynamic changes occur in the bacterial population in the subgingival crevice. The physiochemical properties that develop favor specific species such as *T. denticola*, which transitions from a minor species in the healthy subgingiva to a dominant species in periodontal pockets. The ability to capitalize on the changing environment requires that bacteria be able to sense, read, and respond to nutrient availability, oxygen levels, metabolic by-product concentrations, blood and serum concentrations, pH, and numerous other variables. The molecular mechanisms used by the oral treponemes to drive these adaptive responses have, until recently, been largely undefined. It is our hope that this review will provide a resource that will assist investigators seeking to define the molecular basis of adaptive responses of the oral treponemes. The identification of the key molecular players and mechanisms involved in adaptive responses will open the door for translational studies seeking to develop preventive or treatment strategies for periodontal disease.

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