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REVIEW

Tyrosine phosphorylation and bacterial virulence

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Protein phosphorylation on tyrosine has emerged as a key device in the control of numerous cellular functions in bacteria. In this article, we review the structure and function of bacterial tyrosine kinases and phosphatases. Phosphorylation is catalyzed by autophosphorylating adenosine triphosphate-dependent enzymes (bacterial tyrosine (BY) kinases) that are characterized by the presence of Walker motifs. The reverse reaction is catalyzed by three classes of enzymes: the eukaryotic-like phosphatases (PTPs) and dual-specific phosphatases; the low molecular weight protein-tyrosine phosphatases (LMW-PTPs); and the polymerase–histidinol phosphatases (PHP). Many BY kinases and tyrosine phosphatases can utilize host cell proteins as substrates, thereby contributing to bacterial pathogenicity. Bacterial tyrosine phosphorylation/dephosphorylation is also involved in biofilm formation and community development. The *Porphyromonas gingivalis* tyrosine phosphatase Ltp1 is involved in a restraint pathway that regulates heterotypic community development with *Streptococcus gordonii*. Ltp1 is upregulated by contact with *S. gordonii* and Ltp1 activity controls adhesin expression and levels of the interspecies signal AI-2.
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INTRODUCTION

Regulation of protein activity, as orchestrated by the tightly co-ordinated and balanced dynamics between kinases and phosphatases, is one of the critical determinants of normal cellular growth and development. While the addition or removal of phosphoryl groups to/from serine, threonine or tyrosine residues has long been established as one of the predominant mechanisms of post-translational protein modification in eukaryotes, it was not until the 1970s that landmark studies demonstrated its importance in prokaryotes.[1] Furthermore, protein phosphorylation has now also been shown to be prevalent in archaea.[2] The first characterized phosphorylation systems in prokaryotic organisms were the two component systems (TCS) and the phosphotransferase system (PTS).[3] In the basic configuration of TCS, a surface-exposed sensor kinase is first autophosphorylated in response to an external signal. The phosphoryl group is then transferred to the aspartyl residue of a response regulator, which in turn can modulate gene expression. In this manner, signal transduction is effectuated by phosphate flow. TCS are widespread in bacteria, and they control the response to a wide range of environmental stimuli. Remarkably, any one organism can possess up to 50 functionally isolated systems. In the PTS system, a phosphoryl group from phosphoenol pyruvate is transferred along a chain of proteins by reversible phosphorylation of histidine residues (although HPr is phosphorylated on histidine and serine).[3] The final receptor for the phosphoryl group is a sugar; hence, the PTS is involved in carbohydrate uptake rather than signal transduction.

PHOSPHORYLATION OF PROTEINS IN BACTERIA

Serine and threonine phosphorylation

Bacterial serine/threonine specific phosphorylation was discovered more than 30 years ago, and this post-translational modification is now known to be ubiquitous and is involved in a diverse array of physiological processes including secondary metabolism, catabolite repression, oxidative stress responses and sporulation.[4] Serine/threonine kinases and phosphatases are also involved in bacterial virulence, in particular through their action on host cell substrates (Table 1). For example, the YpkA/YopO kinase of *Yersinia* species is delivered into epithelial cells by type III secretion machinery, whereupon it disrupts actin microfilament structure.[5] Two autophosphorylated Ser/Thr protein kinases, NleH1 and NleH2, in enterohemorrhagic *Escherichia coli*, and the OspG protein in *Shigella flexneri*, inhibit activation of the proinflammatory transcription factor NF-κB.[6–7] The serine phosphatase SerB of *Porphyromonas gingivalis,* which is required for maximal invasion of the organisms into epithelial cells, can impact both the actin and tubulin cytoskeleton of host cells, and also attenuate NF-κB activation.[8–10] The secretion of serine kinases and/or phosphatases has thus afforded bacterial pathogens the means to interfere with host signal transduction pathways.

Tyrosine phosphorylation

The first definitive evidence of protein tyrosine kinase activity in bacteria was discovered in *E. coli* with the identification of phosphotyrosine in partial acid hydrolysates of proteins.[11] Protein tyrosine phosphorylation subsequently was shown to direct many essential

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Table 1 Bacterial kinases and phosphatases involved in virulence through interaction with host cell proteins

Organism	Enzyme	Activity	Impact on host cell function	References
Coxiella burnetii	Acp	Tyrosine phosphatase	Inhibition of human neutrophils	55–56
Enterohemorrhagic	NleH1, NleH2	Ser/Thr kinase	Inhibit activation of NF-kB	6
Escherichia coli	NleH1, NleH2	Ser/Thr kinase	Inhibit activation of NF-kB	6
Listeria monocytogenes	LipA	Tyrosine phosphatase	Actin cytoskeleton disruption	39
Mycobacterium tuberculosis	MPtpA and B	Tyrosine phosphatase	Phagocytosis actin polymerization in macrophages	35, 57
	PknG	Ser/Thr kinase	Inhibition of phagosome-lysosome fusion	58
Porphyromonas gingivalis	SerB	Serine phosphatase	Disruption of actin/tubulin; inhibition of NF-κB activation, intracellular persistence	9–10
Salmonella typhi	StpA	Tyrosine phosphatase	Host cytoskeleton disruption	59
Salmonella 'typhimurium'	SptP	Tyrosine phosphatase	Actin rearrangements	60
Shigella flexneri	OspG	Ser/Thr kinase	Inhibit NF-κB activation	6
	OspF	Dual specific phosphatase	Represses innate immunity	61
Yersinia enterocolitica	YopO	Ser/Thr kinase	Disruption of actin; inhibition of phagocytosis	62
Yersinia pseudotuberculosis	YopH	Tyrosine phosphatase	Cytoskeletal rearrangements; inhibition of phagocytosis	28
Yersinia pseudotuberculosis	YpkA	Ser/Thr kinase	Disruption of actin; inhibition of phagocytosis	5
Yersinia pestis	YpkA	Ser/Thr kinase	Disruption of actin; inhibition of phagocytosis	5

Table 2 Bacterial protein tyrosine kinases and phosphatases and their functional roles

Organism	Tyrosine Kinase	Tyrosine Phosphatase	Substrate(s)	Function	References
Acinetobacter johnsonii	Ptk	Ptp	Ptp uses Ptk as endogenous substrate	Phosphorelay reactions of inner membrane proteins	63
Acinetobacter lwoffii	Wzc	Wzb	Wzb uses Wzc as endogenous substrate	Emulsan production	46
Bacillus subtilis	YwqD, PtkA, PtkB, McsB	YwqE, YfkJ, YwlE, PtpZ	TuaD, Ugd, SsbA, McsA, CtsR, YjoA, YnfE, TvyG, YorK, Asd, YwpH	Exopolysaccharide synthesis, teichuronic acid production, DNA metabolism, heat shock response	64–65
Caulobacter crescentus	DivL	—	CtrA	Cell division	66
Erwinia amylovora	AmsA	AmsI	Lipid carrier di-/monophosphates	Amylovoran production	67
Escherichia coli K-12	Wzc_{CA}	Wzb	Ugd; Wzb uses Wzc as endogenous substrate	Colanic acid synthesis	40, 68
Escherichia coli K-12/K-30	Etk	Etp	RpoH, RseA; Etk	Exopolysaccharide production	69
Escherichia coli K-30	Wzc_{CPS}	Wzb	Ugd	Group 1 capsule assembly	44
Klebsiella pneumonia	Yco6, Wzc	Yor5, Wzb	Yor5 uses Yco6 as endogenous substrate	Capsule synthesis	18, 70
Myxococcus xanthus	MasK	—	MglA	Aggregation, sporulation, motility, development	71
Porphyromonas gingivalis		Ltp1		Exopolysaccharide production, heterotypic community development	49
Pseudomonas aeruginosa	WaaP			Lipopolysaccharide synthesis	72
Pseudomonas aeruginosa	42k		Flagellin a and b proteins;	Flagellin export	73
Pseudomonas aeruginosa		TbpA	Diguanylate cyclase	Exopolysaccharide production, biofilm development	47
Ralstonia solanacearum	EpsB	EpsP		Exopolysaccharide transport	74–75
Salmonella typhimurium	PutA	—	P5C	Proline metabolism	76
Sinorhizobium meliloti	ExoP	—		Succinoglycan production	77
Staphylococcus aureus	Cap5B2	CapC, PtpA, PtpB	Cap5O (UDP-acetyl-mannosamine dehydrogenase)	Capsule synthesis	78
Streptococcus agalactiae	CpsD	CpsB		Polysaccharide chain length	79
Streptococcus pneumoniae	CpsD	CpsB		Capsule synthesis	80
Streptococcus thermophilus	EpsD	EpsB	EpsE	Exopolysaccharide biosynthesis	81
Streptomyces coelicolor A3(2)	AfsK	—	AfsR	Antibiotic production	82–84
	SCO5717			Cell growth	

cellular processes, such as capsule production, growth, proliferation, migration, flagellin export, adaptation to stress and production of secondary metabolites (Table 2).[12] Moreover, the addition of a bulky, negatively charged phosphoryl group to a protein can influence both cellular location and the overall protein interactome.[13] A number of global phosphoproteome studies have now been conducted in bacteria, including *E. coli*, *Helicobacter pylori*, *Bacillus subtilis*, *Streptomyces coelicolor*, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Lactococcus lactis*, *Campylobacter jejuni* and *Pseudomonas species*. These databases have shown an increasing number of bacterial proteins that are phosphorylated on Ser/Thr/Tyr residues; and, moreover, these proteins are involved

in a variety of important cellular functions, including virulence and cell survival.[14–24]

BACTERIAL TYROSINE KINASES

Structure

The bacterial tyrosine (BY) kinase family comprises the major group of bacterial enzymes endowed with tyrosine kinase activity. In most cases, BY kinases possess a transmembrane domain that can function both as an anchor and a sensor, as well as an intracellular catalytic domain.[25] The catalytic domain lacks the distinctive eukaryotic kinase motifs, and is defined by the presence of Walker A (P-loop) and B motifs (Figure 1). In addition, some BY kinases also contain a Walker A′ motif. BY kinases autophosphorylate at a tyrosine rich cluster in the C-terminal region using adenosine triphosphate as a phosphoryl donor, and the degree of phosphorylation in this region determines the interaction strength with other proteins. Some BY kinases also autophosphorylate on a tyrosine residue in close proximity to the Walker A′ box. Recent studies have identified other bacterial tyrosine kinases including those that closely resemble eukaryotic-like kinases, and those that utilize guaidino-phosphotransferase domains. Additionally, in some cases, tyrosine can substitute for histidine in TCS.[25]

Function

The majority of genes encoding BY kinases reside in operons responsible for regulating the synthesis and secretion of polysaccharides. The autophosphorylation state of the BY kinases exerts control over this process through phosphorylation, and activation, of UDP-sugar dehydrogenases and glucosyltransferases.[13] As bacterial regulatory networks are extensively interconnected, the phosphotransfer reactions can modulate a myriad of physiological processes that include resistance to cationic peptides and polymixin, along with heat shock responses. A greater appreciation for the role of tyrosine kinases in prokaryotes has emerged from the application of global phosphoproteome technologies. For example, the PtkA BY kinase of *Bacillus subtilis* can phosphorylate at least nine different protein substrates.[26] Several of these substrates, most notably single-stranded DNA exonuclease YorK and aspartate semialdehyde dehydrogenase Asd, are activated *via* phosphorylation. Yet, the activity of many others, such as enolase, YjoA, YnfE, TvyG, Ugd and SsbA, remains unaffected by phosphorylation, and rather the cellular localization of these proteins is governed by phosphorylation status. Hence, BY action can not only regulate the activity of substrates, but also ensure the correct cellular localization of specific protein targets.

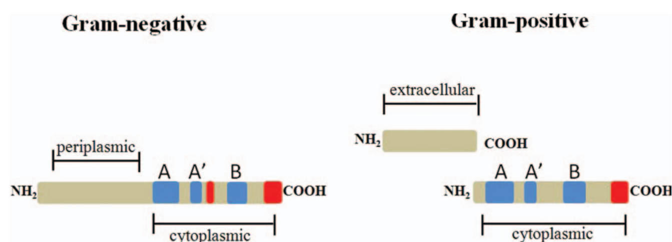

Figure 1 Domain structure of BY kinases. A periplasmic (gram-negatives) or extracellular (gram-positives) sensory loop is linked to the catalytic intracellular domain, either contiguously (gram-negatives) or through protein–protein interaction (gram-negatives). The catalytic domain contains Walker A, B and A′ motifs (blue). A tyrosine-rich region (red) containing the phosphorylation sites is present in the C-terminus, and gram-negative BY kinases also possess an internal tyrosine (red) that can be autophosphorylated. Walker motifs A, A′ and B can be identified by conserved sequences motifs. BY, bacterial tyrosine.

BACTERIAL TYROSINE PHOSPHATASES

Structure

Bacterial tyrosine phosphatases catalyze the dephosphorylation of tyrosyl phosphorylated proteins, which in turn can result in either the propagation or inhibition of phospho-dependent signaling. Bacterial tyrosine phosphatases can be categorized into three distinct families: (i) the eukaryotic-like phosphatases (PTPs) and dual-specific phosphatases that also display activity against phosphoserine and phosphothreonine; (ii) the low molecular weight protein-tyrosine phosphatases (LMW-PTPs), a family of small acidic enzymes also found in eukaryotes; and (iii) the polymerase–histidinol phosphatases (PHP), a family of phosphoesterases commonly found in gram-positive bacteria. The PTP, dual-specific phosphatase and LMW-PTP enzymes utilize a common catalytic mechanism that involves the conserved signature $C(X)_5R$ motif in the phosphate binding loop where cysteine, functioning as a nucleophile, attacks the phosphorus atom of the phosphotyrosine residue of the substrate. The arginine residue interacts with the phosphate moiety of the phosphotyrosine.[27] This motif is flanked, more remotely, by an essential aspartic acid residue, the location of which varies among the families. Protein tyrosine phosphatases also are capable of possessing dual functions, whereby in some instances, they can stimulate actions of cognate protein tyrosine kinases, yet in other cases, they may antagonize those actions.[12] In gram-negative bacteria, the gene encoding the LMW-PTP generally is upstream of the tyrosine kinase in the same operon. Conversely, in gram-positives, a PHP type phosphatase often is located in the same operon as the BY kinase alongside an adaptor protein, with the gene for the LMW-PTP at a remote site.

Function

While bacterial tyrosine phosphatases can be intimately involved in a number of cellular processes, two major themes have become apparent: involvement in polysaccharide production; and as secreted effector proteins with the potential for manipulation of host cell signal transduction pathways. Polysaccharide production, encompassing both exopolysaccharides and capsular polysaccharides, is also a key virulence determinant in many organisms and thus tyrosine phosphatase activity is emerging as a central player in the information flow that controls pathogenic activity.

The YopH protein tyrosine phosphatase of *Yersinia,* a member of the PTP family, is an essential virulence factor that is injected into epithelial cells by type III secretion machinery. YopH can uncouple multiple signal transduction pathways,[28] and in human epithelial cells YopH dephosphorylates several focal adhesion proteins, including p130Cas (Cas), focal adhesion kinase and paxillin.[29–31] Similarly, *Salmonella* '*typhimurium*' translocates the PTP tyrosine phosphatase SptP into epithelial cells where it is involved in reversing mitogen-activated protein kinase activation.[32] SptP is required for full virulence in murine models of disease.[33] *Shigella flexneri* produces a dually specific phosphatase, OspF, that dephosphorylates mitogen-activated protein kinase, which consequently prevents histone H3 phosphorylation.[34]

A reduction in the level of histone 3 phosphorylation impedes access of the transcription factor NF-κB to the chromosome and hence transcription of NF-κB responsive genes such as IL-8 is reduced. Thus, OspF activity allows *S. flexneri* to modulate host cell epigenetic information as a strategy for repressing innate immunity. *Mycobacterium tuberculosis* secretes two LMW-PTPs, PtpA and PtpB.[35] The predicted lack of tyrosine kinases in the *M. tuberculosis* genome suggests a dedicated role for these phosphatases in regulation of host cell functions. Expression of *ptpA* in *M. tuberculosis* is upregulated within monocytes, and a *ptpB* mutant is impaired in its ability to grow in human macrophages[36] and survive in a guinea pig model.[37] These phosphatases appear to function by impacting actin polymerization within macrophages and thereby affecting phagocytosis of the organism.[38] A recently identified phosphatase, LipA, in *L. monocytogenes* has a predicted structure bearing a remarkable semblance to the PtpB phosphatase.[39] Moreover, both LipA and PtpB share a unique feature whereby they possess dual-function activities as phosphotyrosine and phosphoinositide phosphatases, and both harbor the potential to play pivotal roles in bacterial virulence.

In addition to physical protection, exopolysaccharide such as capsule is often poorly immunogenic and can mask protein antigens and receptors for complement and phagocytic cells. In many cases, dephosphorylation of tyrosine kinases increases the level of polysaccharide synthesis,[4] as evidenced by the activity of the *E. coli* K-12 BY kinase Wzc-ca, which is regulated by its cognate LMW-PTP, Wzb.[40] In this system, production of the capsular exopolysaccharide colonic acid is maximal when Wzc-ca is dephosphorylated by Wzb. Similarly, in *Streptococcus pneumoniae* autophosphorylation of the CpsD kinase, when in the presence of its cognate partner, CpsC, results in the attenuation of CpsD kinase activity, as well as a reduction in the level of encapsulation *via* a negative feedback regulatory loop.[41] Consequently, the PHP family phosphatase CpsB can control capsule production *via* dephosphorylation of CpsD which functions as a reversible switch.[42] The converse situation also exists. In clinical isolates of *S. pneumoniae,* phosphorylation of CpsD increases capsule production under anaerobic conditions,[43] and in *E. coli* K30 the assembly of group I capsular polysaccharides is elevated by phosphorylation of Wzc-cps.[44] Undoubtedly, the interplay among tyrosine kinases, phosphatases and exopolysaccharide is of a nuanced and subtle nature that may be reconfigured according to environmental conditions. Indeed, metabolic activity is one factor that has been shown to influence kinase to phosphatase ratios.[45–46]

Role in biofilms

A recent study in *Pseudomonas aeruginosa* demonstrated that tyrosine phosphatase activity is a unifying element that amalgamates polysaccharide production and biofilm formation with quorum sensing.[47] The PTP family tyrosine phosphatase, TpbA, is a negative regulator of 3,5-cyclic diguanylic acid (c-di-GMP), an important second messenger which suppresses transcription across the *pel* operon that encodes for extracellular matrix polysaccharide. Lower levels of exopolysaccharide in turn lead to reduced biofilm formation. In addition, TpbA responds to acyl homoserine lactone, and *tpbA* is regulated positively by the LasR transcriptional regulator. TpbA also regulates cell lysis as a means to control extracellular DNA that is used for complex biofilm maturation.[48] These findings also reveal a previously unrecognized ability for phospho-dependent signaling to intersect with other important cellular second messenger systems.

Tyrosine phosphatases can also control heterotypic biofilm formation among oral organisms. *P. gingivalis* accumulates into heterotypic communities with the antecedent oral biofilm colonizer *S. gordonii* (Figure 2). Maeda *et al.*[49] identified a LMW-PTP, Ltp1, in *P. gingivalis* which functions as a negative regulator of EPS production, as well as community formation with *S. gordonii*. Transcription of *ltp1* is increased following contact with *S. gordonii*,[50] and Ltp1 is a component of a signaling pathway that converges on the LuxR family transcriptional regulator CdhR.[51] The expression of the *P. gingivalis* Mfa fimbriae and of LuxS, both of which contribute to community development with *S. gordonii*, are negatively regulated by CdhR (Figure 3). Thus, in both *P. aeruginosa* and *P. gingivalis*, tyrosine phosphatase activity results in arrested community development which may maintain optimal biofilm architecture.

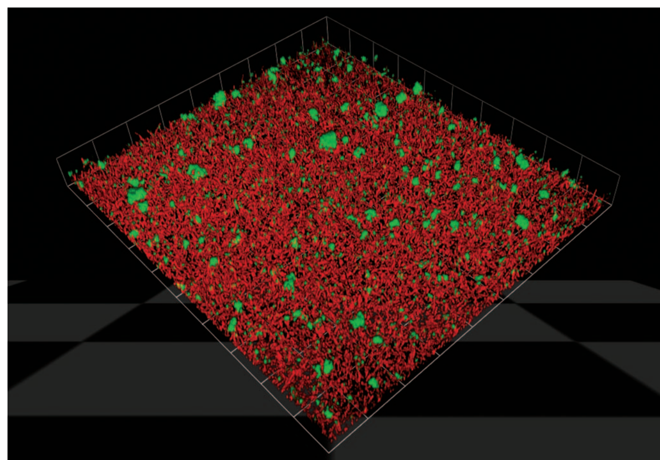

Figure 2 ***Porphyromonas gingivalis*** **(green) accumulates into a mixed species community on a substratum of** ***Streptococcus gordonii*** **(red).** Image courtesy of Dr Christopher Wright.

CONCLUSION

It is sobering to reflect that until fairly recently, post-translational modification of tyrosine residues by phosphorylation was believed to be an indicator of the sophisticated regulatory networks characteristic of eukaryotic systems. Since then, research on bacterial tyrosine kinases and phosphatases has proceeded apace and they are now considered key contributors to bacterial cell homeostasis, virulence and even cell survival in all domains of life. Future genomics and proteomics research will decipher and dissect the underlying mechanisms of tyrosine phosphotransfer cascades in bacteria and their functional roles. Accumulating evidence reaffirms the notion that bacterial tyrosine kinases and phosphatases display exquisite substrate specificity; nevertheless, they are still capable of utilizing multiple protein substrates, both endogenous and exogenous, thereby providing versatility in phosphorelay signaling networks. Ongoing studies reveal increasing instances where bacterial kinases/phosphatases are capable of inducing post-translational modifications of host proteins and are a crucial facet of the dynamic host-pathogen relationship. Moreover, as BY kinases differ from their eukaryotic counterparts in significant biochemical and structural aspects, they provide attractive targets for specific antibacterial drugs. The crystal structures of the CapB kinase of *Staphylococcus aureus* and the Etk kinase of *E. coli* have been determined[52–53] and they exhibit a high degree of structural similarity. The availability of structural and biochemical information will facilitate the rational design of compounds that can inhibit BY kinases, while concomitantly avoiding any eukaryotic kinases.[54]

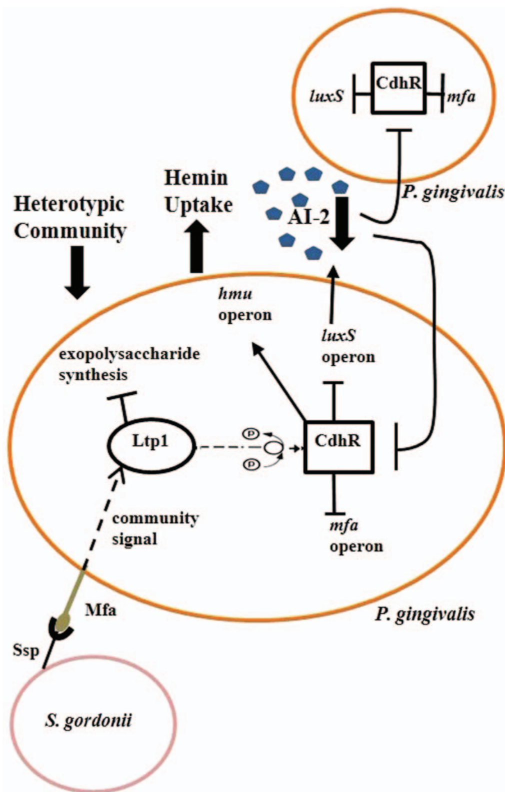

Figure 3 Model of the tyrosine phosphatase-dependent regulatory circuitry governing heterotypic community development between *Porphyromonas gingivalis* and *Streptococcus gordonii.* Initial interaction of the *P. gingivalis* Mfa fimbriae with *S. gordonii* activates the Ltp1 phosphatase and a signaling event is transduced *via* a cascade of phosphorylation/dephosphorylation events. Signaling converges on CdhR which represses transcription of the *luxS* and *mfa* operons in *P. gingivalis*, and in turn leads to constrained *P. gingivalis*–*S. gordonii* community development. Lower AI-2 levels can be sensed by neighboring planktonic *P. gingivalis* cells, which also upregulate CdhR, thereby propagating the original streptococcal-derived signal throughout the *P. gingivalis*–*S. gordonii* community. (Modified from Molecular Microbiology 2011; 81(2): 305–314; this material is reproduced with permission of John Wiley & Sons, Inc.)

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