environmental microbiology

Environmental Microbiology (2010) **12**(8), 2051–2059 doi:10.1111/j.1462-2920.2010.02269.x

Minireview

Control of biofilm formation and colonization in *Vibrio fischeri*: a role for partner switching?

Andrew R. Morris and Karen L. Visick*
Department of Microbiology and Immunology, Loyola University Medical Center, Maywood, IL, USA.

Summary

Bacteria employ a variety of mechanisms to promote and control colonization of their respective hosts, including restricting the expression of genes necessary for colonization to distinct situations (i.e. encounter with a prospective host). In the symbiosis between the marine bacterium *Vibrio fischeri* and its host squid, *Euprymna scolopes*, colonization proceeds via a transient biofilm formed by the bacterium. The production of this bacterial biofilm depends on a complex regulatory network that controls transcription of the symbiosis polysaccharide (*syp*) gene locus. In addition to this transcriptional control, biofilm formation is regulated by two proteins, SypA and SypE, which may function in an unusual regulatory mechanism known as partner switching. Best characterized in *Bacillus subtilis* and other Gram-positive bacteria, partner switching is a signalling mechanism that provides dynamic regulatory control over bacterial gene expression. The involvement of putative partner-switching components within *V. fischeri* suggests that tight regulatory control over biofilm formation may be important for the lifestyle of this organism.

Introduction

To achieve efficient colonization of their respective hosts, bacteria have evolved complex signalling networks to ensure the proper expression of the genes necessary to respond to the host environment. The result of these regulatory cascades is the induction of key cellular responses required for successful host colonization, such as biofilm formation. Biofilms, or surface-associated communities of cells encapsulated in a matrix, often play an integral role in the attachment of bacterial cells to host or environmental surfaces and in bacterial survival, both within and outside of a host.

The formation of a biofilm is a common strategy utilized among numerous *Vibrio* species, in which it is predicted to promote bacterial persistence in the environment and/or colonization of eukaryotic hosts (for a recent review see Yildiz and Visick, 2009). *Vibrio* species are Gram-negative bacteria, typically present in marine environments. Among the *Vibrios*, several species engage in pathogenic or symbiotic partnerships with specific eukaryotic hosts. This review focuses on the bacterium *Vibrio fischeri*, as recent work has shown a clear relevance of biofilm formation to the ability of this organism to establish symbiotic colonization of its eukaryotic host, the Hawaiian bobtail squid *Euprymna scolopes*. Biofilm formation by *V. fischeri* requires expression of a cluster of polysaccharide biosynthetic genes, termed the symbiosis polysaccharide (*syp*) locus, that is conserved among several *Vibrio* species (Yip *et al.*, 2005; 2006). Importantly, the regulation of this polysaccharide locus involves an intricate network of regulatory proteins, which is predicted to restrict biofilm formation such that it occurs transiently in response to specific host-derived signals (Visick, 2009).

Biofilm formation also appears to be regulated by two largely uncharacterized regulatory proteins encoded within the *syp* cluster, SypA and SypE. Bioinformatic analyses of these regulatory proteins suggest they contain elements of a regulatory signalling mechanism, termed partner switching. A signalling mechanism most extensively characterized in Gram-positive bacteria, partner switching provides yet another layer of regulatory control over gene expression. In recent years, genome analyses have suggested that potential partner-switching components are present in a wide range of bacteria, including Gram-negative species (Mittenhuber, 2002; Mattoo *et al.*, 2004). Here, following brief overviews of the *V. fischeri*–squid symbiosis and biofilm formation, we review the partner-switching mechanism, as understood within characterized models, then speculate on the role of

Received 24 February, 2010; accepted 20 April, 2010. *For correspondence. E-mail kvisick@lumc.edu; Tel. (+1) 708 216 0869; Fax (+1) 708 216 9574.

this signalling mechanism in the regulation of biofilms by *V. fischeri*, and potentially, other *Vibrio* species.

Vibrio fischeri and *E. scolopes*: symbiotic initiation depends on biofilm formation

The symbiotic relationship between the marine bacterium *V. fischeri* and its eukaryotic host, *E. scolopes*, provides an elegant model of symbiotic bacteria–host interaction (for recent reviews, see Nyholm and McFall-Ngai, 2004; Stabb, 2006; Visick and Ruby, 2006). Newly hatched juvenile squid are aposymbiotic and must acquire their bacterial symbionts from the surrounding seawater. Successful establishment of this symbiotic colonization involves a number of both host- and symbiont-derived responses (Nyholm and McFall-Ngai, 2004). Importantly for this review, exposure to environmental bacteria stimulates newly hatched squid to secrete mucus onto the surface of their symbiotic light organs (Nyholm *et al*., 2000; Nyholm and McFall-Ngai, 2004). *Vibrio fischeri* appears particularly adept at adhering to the mucus and forming a biofilm-like aggregate of cells that are poised to enter the light organ (Nyholm and McFall-Ngai, 2003). Subsequently, *V. fischeri*, but not other bacteria, productively enter and migrate to the crypts, where they establish colonization by multiplying to high cell density.

The formation of a biofilm aggregate outside the squid light organ is essential for efficient initiation of host colonization. Mutants defective in biofilm formation exhibit a significant defect in colonization, while a strain with an enhanced ability to form biofilms exhibits a significant colonization advantage (Yip *et al*., 2005; 2006). Formation of this biofilm requires the *syp* locus, consisting of 18 genes predicted to be involved in the synthesis and regulation of a polysaccharide biofilm matrix (Yip *et al*., 2005; 2006). *syp* mutants exhibit a significant defect in biofilm formation and host colonization (Yip *et al*., 2005).

Regulation of biofilm formation: a complex network of regulators

Biofilm formation appears to be under complex regulatory controls. At least four regulators encoded within the *syp* locus (SypA/E/F/G) and two regulators encoded elsewhere (RscS and VpsR) appear to regulate biofilms at the level of *syp* transcription or at an unknown level beyond *syp* activation (recently reviewed in Visick, 2009). Transcription of the *syp* locus is controlled by the response regulator SypG, which is predicted to be activated via phosphotransfer from an upstream sensor kinase, RscS (Fig. 1) (Hussa *et al*., 2008). Overexpression of either *rscS* or *sypG* promotes substantial biofilm formation, and loss of either gene results in a severe colonization defect similar to *syp* mutants (Visick and Skoufos, 2001; Yip

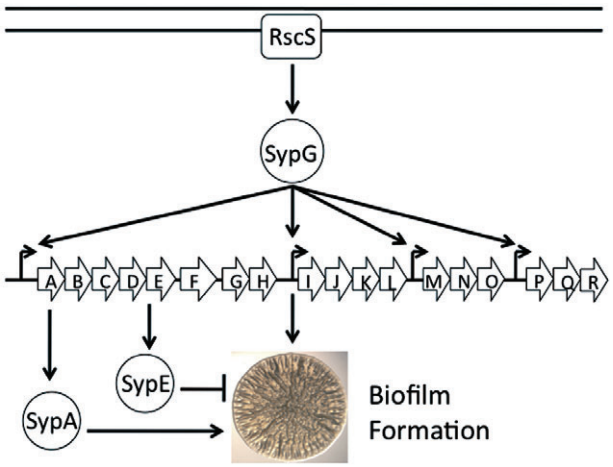

Fig. 1. Model of biofilm formation in *V. fischeri*. The symbiosis polysaccharide (*sypA–R*) locus is regulated at the transcriptional level via a two-component regulatory cascade consisting of the sensor kinase, RscS, and the downstream response regulator, SypG. The regulatory proteins, SypA and SypE, exhibit antagonistic regulatory roles, promoting and inhibiting *syp*-dependent biofilms respectively. These regulators control biofilm formation via an unknown mechanism that appears to function downstream of *syp* transcription. Biofilms are represented by the formation of a wrinkled bacterial colony (Yildiz and Visick, 2009).

et al., 2006; Hussa *et al*., 2007). Two other regulators, the sensor kinase SypF and the response regulator VpsR (a predicted DNA-binding protein), also appear to regulate biofilm formation, but it remains unknown how these proteins contribute to the regulatory network (Darnell *et al*., 2008).

SypA and SypE also contribute to control of biofilm formation, but appear to exert their effects downstream of *syp* transcription (Hussa *et al*., 2008; A.R. Morris and K.L. Visick, unpubl. data). Current unpublished data indicate the SypA is required for biofilm formation by *V. fischeri* (S. Shibata, E.S. Yip and K.L. Visick, unpubl. data). Sequence analysis indicates that *sypA* codes for a single-domain protein with a predicted sulfate transporter and anti-sigma factor antagonist (STAS) domain (Fig. 2). This domain is conserved among anti-anti-sigma factors, which generally function as positive regulators (Aravind and Koonin, 2000).

In contrast to SypA, SypE appears to play a dual role in biofilm formation. SypE inhibits SypG-induced biofilm formation, but is required for full biofilm induction under conditions in which the sensor kinase, RscS, is overexpressed (Hussa *et al*., 2008). Sequence and domain analysis of SypE suggests a unique multi-domain protein. Due to the presence of a conserved receiver (REC) domain, SypE is a predicted response regulator. The activity of response regulators is generally controlled via the phosphorylation of a conserved aspartate residue within the REC domain (Stock *et al*., 2000). The

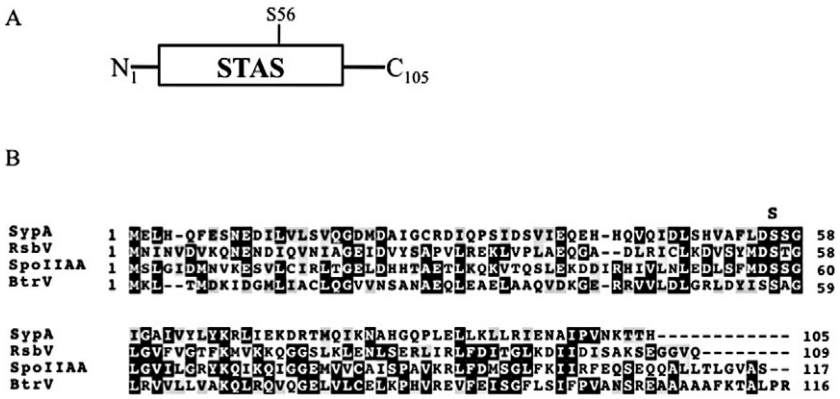

Fig. 2. SypA domain structure and multiple sequence alignment.
A. Domain structure of SypA. SypA contains the conserved anti-sigma factor antagonist and sulfate transporter (STAS) domain present in anti-anti-sigma factors. The conserved regulatory serine residue (S56) is indicated.
B. BLAST multiple sequence alignment (Altschul *et al*., 1997) and identification of conserved residues within *V. fischeri* SypA and the anti-anti-sigma factors RsbV and SpoIIAA of *B. subtilis* and BtrV of *B. bronchiseptica*. Serine 56 of RsbV, serine 58 of SpoIIAA and serine 59 of BtrU are the phosphorylation targets of the respective serine kinases (Najafi *et al*., 1995; Yang *et al*., 1996). This serine residue, indicated by an S, is conserved in SypA (S56). Highlighting of the conserved identical residues (black boxes) and conserved substitutions (grey boxes) was generated using BOXSHADE server.

phosphorylation state of the REC domain activates an effector domain within the response regulator that results in a response, usually a change in gene transcription (Stock *et al*., 2000). Distinct from the typical response regulator, which contains the REC domain in the N-terminus, SypE contains a central REC domain (Fig. 3A). This central REC domain is flanked by two domains of putative opposing functions. The N-terminal domain of SypE exhibits weak sequence similarity to serine kinases of the GHKL (gyrase, Hsp90, histidine kinase and MutL) family, which includes SpoIIAB of *Bacillus subtilis* (Dutta and Inouye, 2000) (Fig. 3B). The C-terminus of the protein contains a putative serine/threonine phosphatase domain and exhibits strong sequence similarity to the PP2C family of serine phosphatases (Fig. 3C).

How these proteins contribute to the biofilm regulatory network remains an area of active research. Although the roles of SypA and SypE in the regulation of biofilm formation remain unknown, bioinformatics suggest that these proteins may constitute a novel partner-switching system.

The partner-switching system

First coined by Alper and colleagues (1994), the term 'partner switch' describes a network of interacting proteins whose interaction with cognate 'partners' depends upon a reversible phosphorylation event. Dependent upon which partner proteins interact, the outcome of this partner switching can either negatively or positively regulate a target protein, generally a transcription factor or enzyme. Specifically, the partner-switching mechanism depends upon key regulatory elements, including a serine kinase/anti-sigma factor, a serine phosphatase, an antagonist protein/anti-anti-sigma factor and a target protein (often a sigma factor) (Yang *et al*., 1996).

The partner-switching mechanism has been best characterized in the Gram-positive bacterium *B. subtilis*. In *B. subtilis* and other Gram-positive bacteria, partner-switching systems contribute to the signalling networks that control the activity of sigma factors, the subunit of RNA polymerase that provides promoter specificity. For example, *B. subtilis* uses partner switching to control the activity of the sigma factor, sigma B, which regulates the general stress response pathway (Dufour and Haldenwang, 1994). In this system, the anti-sigma factor RsbW negatively regulates the activity of sigma B by directly binding it and preventing interaction with the RNA polymerase core (Dufour and Haldenwang, 1994; Hughes and Mathee, 1998) (Fig. 4A). The binding of an anti-anti-sigma factor, RsbV, to RsbW relieves this negative regulation by sequestering the anti-sigma factor (Dufour and Haldenwang, 1994). This releases sigma B, which can now bind to the RNA polymerase core and promote transcription of sigma B-dependent genes. This results in the induction of proteins involved in protection against multiple bacterial stresses (Yang *et al*., 1996; Price *et al*., 2001).

Activity of the anti-anti-sigma factor RsbV is controlled via phosphorylation of a conserved serine residue within the STAS domain (Figs 2B and 4A). In the unphosphorylated state, RsbV can bind and inactivate the anti-sigma RsbW, thus freeing sigma B and upregulating sigma B-dependent genes. However, when this serine residue becomes phosphorylated, RsbV is rendered unable to bind RsbW, which permits RsbW to sequester sigma B

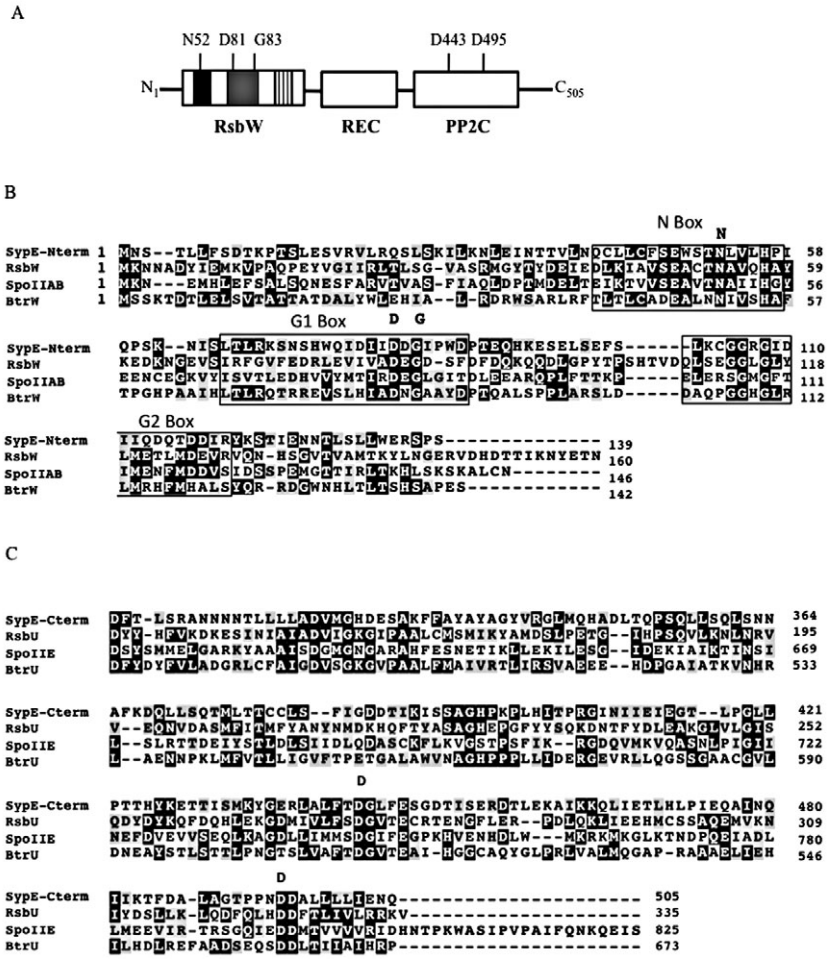

Fig. 3. SypE domain structure and multiple sequence alignment.
A. Domain structure of SypE. SypE is a multi-domain protein that contains a central response regulator (REC) domain flanked by an N-terminal serine kinase (RsbW) domain and a C-terminal serine phosphatase (PP2C) domain. The N-terminal RsbW domain of SypE contains the conserved N-, G1- and G2-boxes important in anti-sigma factor activity, which are indicated by black, grey and striped boxes respectively. The conserved residues within the N-terminal RsbW and C-terminal PP2C domains, predicted to be important for serine kinase or serine phosphatase activity, are shown.
B. BLAST multiple sequence alignment (Altschul *et al*., 1997) of the N-terminal serine kinase domain of SypE with the anti-sigma factors RsbW and SpoIIAB of *B. subtilis* and BtrW of *B. bronchiseptica*. The conserved N-, G1- and G2-boxes are outlined, and the conserved residues required for serine kinase activity are indicated in bold letters above the alignments (Dutta and Inouye, 2000). The SypE serine kinase domain contains the conserved N-box asparagine (N52) and the G1-box aspartate and glycine residues (D81 and G83).
C. BLAST multiple sequence alignment of the C-terminal serine phosphatase domain of SypE with the serine phosphatases RsbU and SpoIIE of *B. subtilis* and BtrU of *B. bronchiseptica*. The labelled amino acids indicate the conserved residues required for serine phosphatase activity (Adler *et al*., 1997). SypE contains the invariant aspartate residues (D443 and D495) predicted to be important in divalent cation binding.
For (B) and (C), highlighting of the conserved residues (black boxes) and conserved substitutions (grey boxes) was generated using BOXSHADE server.

and downregulate sigma B-dependent gene expression. The phosphorylation state of this serine residue is controlled by two sets of proteins, one of which is RsbW itself; in addition to its role in sequestering sigma B, RsbW also functions as a serine kinase. Dephosphorylation of this residue is carried out by the serine phosphatases, RsbU and RsbP, which are activated by environmental and energy stress signals respectively (Voelker *et al*., 1996). Thus, in this regulatory network, RsbW functions as a regulatory switch, as it reversibly interacts with its cognate sigma factor and anti-anti-sigma factor. Furthermore, the partner switch is regulated by reversible phosphorylation of RsbV.

Conservation of partner switching: a Gram-positive mechanism?

Since its characterization in *B. subtilis*, the partner-switching mechanism has been identified as a signalling component in a wide range of Gram-positive bacteria.

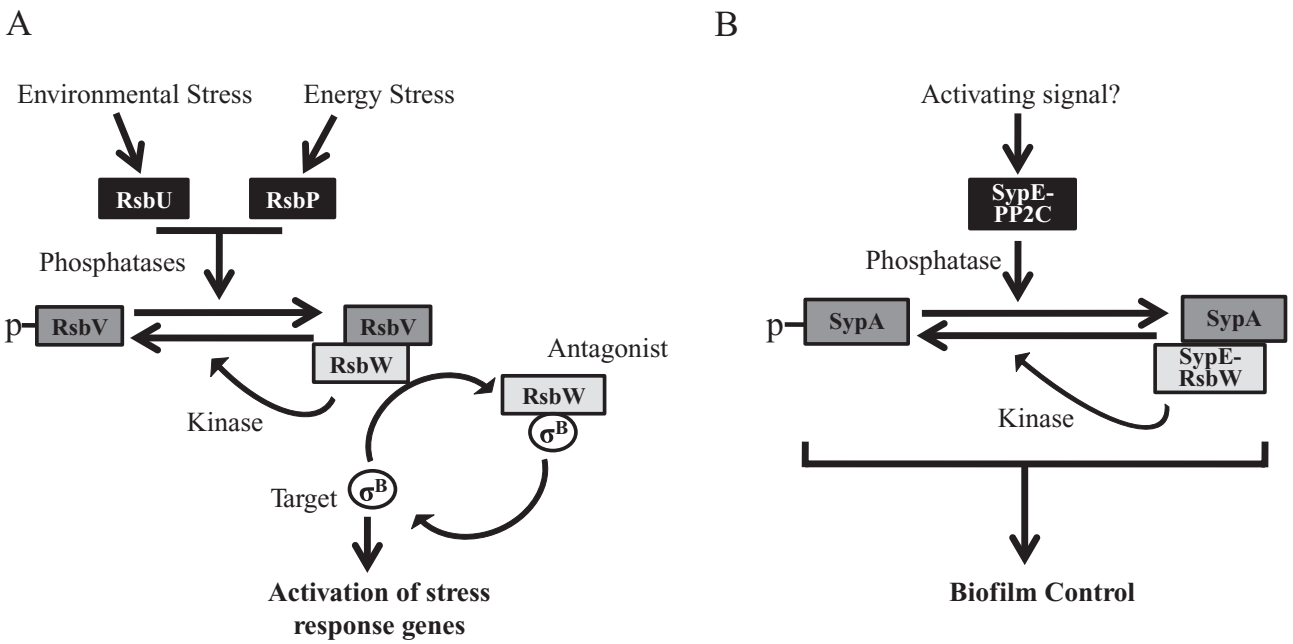

Fig. 4. Model of partner-switching pathways.
A. A model of the *B. subtilis* partner-switching regulatory pathway controlling the activity of the general stress response sigma factor, sigma B. See text for detailed description of the model.
B. A model of the predicted SypA–SypE partner-switching module controlling biofilm formation. SypA and SypE possess the core components of a putative partner-switching signal pathway. The proposed model is constructed from information of the conserved protein domains and current data of biofilm regulation.

These include *Bacillus cereus* (van Schaik *et al*., 2005), *Bacillus anthracis* (Fouet *et al*., 2000), *Mycobacterium tuberculosis* (Beaucher *et al*., 2002), *Staphylococcus aureus* (Miyazaki *et al*., 1999) and *Listeria monocytogenes* (Chaturongakul and Boor, 2004; 2006). In these bacterial systems, partner-switching modules are utilized in a manner similar to that observed in *B. subtilis*: primarily, the regulation of sigma factor activity. However, the output of these modules varies among the individual bacteria. Partner-switching modules have been demonstrated to contribute to the regulation of the general stress response of *L. monocytogenes* and many other bacteria (Chaturongakul and Boor, 2004), biofilm formation in *Staphylococcus epidermidis* (Knobloch *et al*., 2004), and the expression of virulence-associated genes in *M. tuberculosis* (Beaucher *et al*., 2002; Manganelli *et al*., 2004). Additionally, bacteria may possess multiple partner-switching pathways regulating distinct sets of target proteins. For example, in addition to the RsbU/V/W module that regulates the general stress response, *B. subtilis* possesses a second set of partner-switching regulators, SpoIIAA/SpoIIAB/SpoIIE. Similar to the sigma B regulatory pathway, these partner-switching proteins control the activity of a sigma, in this case sigma F, which regulates sporulation (Magnin *et al*., 1997). The partner-switching mechanism, while similar among diverse Gram-positives, has been adapted to respond to specific stimuli and regulate distinct cellular responses.

Partner switching within the Gram-negatives?

Analyses of diverse bacterial genomes suggest that partner-switching orthologues may exist in a wide range of eubacteria (Mittenhuber, 2002; Mattoo *et al*., 2004). Despite its predicted widespread distribution, partner switching has remained relatively uncharacterized within Gram-negative bacteria. Indeed, partner-switching systems have been characterized in only two Gram-negative bacteria, *Bordetella bronchiseptica* and *Chlamydia trachomatis* (Kozak *et al*., 2005; Hua *et al*., 2006). Furthermore, only in the case of *B. bronchiseptica* has a partner-switching module been experimentally demonstrated to regulate a physiological response. This respiratory pathogen utilizes a partner-switching module to control production of a type III secretion system (T3SS) (Mattoo *et al*., 2004). The T3SS consists of a needle-like secretory apparatus that directly transports virulence proteins into the cytoplasm of host cells. In *B. bronchiseptica*, the T3SS contributes to persistent colonization of the host trachea and the avoidance of the host immune response (Yuk *et al*., 2000; Mattoo *et al*., 2001). The production of the T3SS requires transcription of a gene cluster, the *bsc*

locus, which encodes multiple components of the secretory system (Mattoo *et al*., 2001). Regulation of the T3SS depends upon a set of genes adjacent to the *bsc* cluster, the *btr* locus, which encode orthologues of the *B. subtilis* RsbU/V/W partner-switching proteins, BtrU/BrtV/BtrW. *In vitro* and *in vivo* analyses of the *B. bronchiseptica* proteins demonstrated that they constitute a regulatory network similar to their *B. subtilis* counterparts (Kozak *et al*., 2005). However, this partner-switching system appears to deviate from that of the *B. subtilis* RsbU/V/W paradigm. First, disruption of any component of the BtrU/V/W partner-switching module results in the loss of type III secretion (Mattoo *et al*., 2004), a result that is inconsistent with the *B. subtilis* model (Fig. 4A). Second, positive regulation of the T3SS requires both the formation of the BtrV/BtrW complex and its dissociation, via phosphorylation of BtrV by BtrW (Kozak *et al*., 2005). Finally, although the BtrU/V/W module regulates type III secretion, it does not appear to control transcription of the *bsc* locus (Kozak *et al*., 2005). Instead, Kozak and colleagues (2005) suggest that these partner-switching proteins may regulate the T3SS at the posttranscriptional level possibly by interacting with yet unknown regulatory proteins or playing a structural role in the secretory pathway. Thus, although there is conservation of the partner-switching components, the regulatory mechanism appears to vary from that of the Gram-positive paradigm.

Genome analysis of the obligate intracellular pathogen *C. trachomatis* identified several components of a putative partner-switching module (Hua *et al*., 2006). *In vitro* analysis of the candidate genes demonstrated that these proteins could interact. As with *B. bronchiseptica*, it appears that the *C. trachomatis* partner-switching system may vary from the *B. subtilis* paradigm, as *in vitro* binding assays failed to demonstrate an interaction with any of the three sigma factors encoded in the *C. trachomatis* genome (Hua *et al*., 2006). However, the lack of genetic tools and difficulty in culturing *C. trachomatis* have delayed analysis of this potential partner-switching module *in vivo*.

Together, these studies suggest that the partner-switching mechanism, previously observed only among the Gram-positives, also contributes to regulatory control in Gram-negative bacteria. It remains unknown how these Gram-negative partner-switching proteins regulate downstream targets. Furthermore, it remains unclear how widespread this regulatory mechanism is among Gram-negative bacteria.

SypA and SypE: a potential partner-switching module in *V. fischeri*?

Several lines of evidence suggest that SypA and SypE may represent a partner-switching module in the Gram-negative bacterium *V. fischeri*. First, the physical proximity of the *sypA* and *sypE* genes and their known roles in biofilm formation suggest a regulatory connection. Second, searches for *V. fischeri* proteins with sequence similarity to the RsbU/V/W of *B. subtilis*, and other orthologues, yielded only two candidate proteins, SypA and SypE. Third, these proteins not only contain the conserved domains, but also critical active-site residues within these domains.

Specifically, SypA not only contains the conserved STAS domain present among RsbV-like anti-anti-sigma factors, but also retains the conserved serine residue (S56) predicted to be the site of phosphorylation (Fig. 2B). Our preliminary data are consistent with a role for this residue in SypA function (A.R. Morris and K.L. Visick, unpublished). SypE contains two partner-switching domains: an N-terminal serine kinase (RsbW) and a C-terminal serine phosphatase (PP2C) domain. As shown in Fig. 3B, sequence alignment of the N-terminal domain of SypE with RsbW orthologues indicates that SypE contains the conserved N-, G1- and G2-boxes present in the Bergerat ATP-binding fold of serine kinases (Dutta and Inouye, 2000). Importantly, SypE possesses the invariant asparagine residue (N52) of the N-box, which is required for Mg^{2+} ion chelation and coordinates binding of ATP to the nucleotide pocket (Fig. 3B) (Dutta and Inouye, 2000). Sequence alignment also reveals the conserved G1-box aspartate and glycine residues (D81 and G83), predicted to participate in ATP binding and formation of the ATP lid of the nucleotide binding pocket respectively (Dutta and Inouye, 2000). The conservation of these key residues suggests that, despite poor overall sequence similarity, the N-terminus of SypE may function as an RsbW-like serine kinase. The C-terminus of SypE possesses strong sequence similarity to serine/threonine phosphatases of the PP2C family, including RsbU. As shown in Fig. 3C, the C-terminal domain of SypE also possesses the invariant aspartate residues (D306, D323, D443 and D495) that form part of the catalytic core and are predicted to coordinate binding of Mg^{2+}/Mn^{2+} ions necessary for PP2C catalytic activity (Adler *et al*., 1997; Rantanen *et al*., 2007; Shi, 2009).

The antagonistic domains within SypE suggest that this protein may possess both negative and positive regulatory activity, depending on which domain is active (Fig. 4B). Studies of *syp*-dependent biofilms support this hypothesis: SypE inhibits SypG-induced biofilms, but is required for full expression of biofilms produced by overexpression of the sensor kinase, RscS (Hussa *et al*., 2008). Furthermore, recent genetic analyses indicate that the antagonistic domains of SypE may be responsible for these apparent dual regulatory activities (A.R. Morris and K.L. Visick, unpubl. data).

Based on bioinformatics and experimental observations, we propose a model in which SypE and SypA

constitute part of a partner-switching module (Fig. 4B). In partner-switching systems, RsbW-like serine kinases generally function as antagonists, and inhibit cellular responses via the binding to cognate partner proteins. Thus, potentially the N-terminal (RsbW) domain of SypE regulates biofilm formation by interacting with SypA or a yet unknown regulatory protein. These interactions are possibly dictated by the phosphorylation state of SypA, which may be controlled by the serine kinase and/or the serine phosphatase domains of SypE. The phosphorylation state of the central REC domain may regulate whether SypE functions as a serine kinase or a serine phosphatase. The outcome of this partner-switching network is either the negative or the positive regulation of biofilm formation. Work is currently in progress to assess whether these components indeed function as predicted by this model.

Conservation of SypA and SypE among *Vibrio* species

The *syp* locus is relatively conserved among several *Vibrio* species, including both pathogenic and symbiotic bacteria. Although biofilm formation has been investigated in diverse *Vibrio* species, the role of the *syp* cluster in species other than *V. fischeri* is not fully understood. Recently, Kim and colleagues (2009) demonstrated that the *syp* locus plays a role in biofilms formed by the pathogenic bacterium *Vibrio vulnificus.* Specifically, the *syp* cluster contributes to the production of exopolysaccharides and bacterial attachment to biotic and abiotic surfaces (Kim *et al*., 2009). Due to the conservation of the *syp* locus among *Vibrio* species, we performed a bioinformatic survey of *syp*-containing *Vibrio* genomes for SypA and SypE, specifically, or other potential partner-switching proteins. Interestingly, SypA is well conserved among the *syp*-containing *Vibrio* genomes, but SypE appears to be absent in several species. For example, *V. vulnificus* possesses a SypA orthologue, but lacks any clear SypE-like genes. Intriguingly, the genome of *V. vulnificus* contains RsbW- and RsbU-like proteins, VVA0582 and VVA1682, which encode a putative RsbW-like anti-sigma factor and an RsbU-like serine phosphatase respectively. The genomes of *Vibrio parahaemolyticus* and *Aliivibrio salmonicida* similarly lack SypE, but encode RsbW and RsbU-like proteins elsewhere. Thus, in several *Vibrio* species lacking SypE, other putative partner-switching components exist; whether or not they function as predicted or control the activity of SypA remains to be determined.

Concluding remarks

The symbiotic bacterium *V. fischeri* employs a variety of regulatory proteins to control biofilm formation and consequently host colonization. While much of the regulation occurs via canonical two-component signalling pathways that control *syp* transcription, it remains unknown how the *syp*-encoded regulators SypA and SypE control biofilm formation. Bioinformatics suggest the possibility that SypA and SypE participate in a partner-switching mechanism. However, the downstream target of this potential control mechanism remains unknown. In contrast to the well-established Gram-positive partner-switching paradigms, studies from other Gram-negative bacteria suggest that the downstream target may not necessarily be a sigma factor.

The potential integration of partner-switching components within the *V. fischeri* biofilm regulatory network would provide yet another layer of signal control. Together, these mechanisms may confine expression of colonization genes to defined conditions (i.e. interaction with juvenile host squid) and/or control the timing of the transient biofilm formation, thus permitting cells to leave the biofilm to enter the symbiotic organ. The presence of the *syp* locus within multiple *Vibrio* species exhibiting varied lifestyles (pathogens versus symbionts) suggests that *syp*-dependent biofilms may play a role in diverse responses, such as the colonization of respective hosts. The elucidation of the roles of SypA and SypE and the signals to which they respond in *V. fischeri* thus has the potential to provide a paradigm for understanding partner switching in *Vibrios* and other Gram-negatives.

Acknowledgements

We thank Valerie Ray, Satoshi Shibata, Justin Eddy, Bruno Lima, Michael Misale, Jon Visick and Alan Wolfe for their comments on the manuscript. Work on SypA and SypE discussed in this review was supported in part by NIH R01 Grant GM59690 awarded to K.L.V. and a grant awarded to A.R.M. by the Conservation Medicine Center of Chicago.

References

Adler, E., Donella-Deana, A., Arigoni, F., Pinna, L.A., and Stragler, P. (1997) Structural relationship between a bacterial developmental protein and eukaryotic PP2C protein phosphatases. *Mol Microbiol* **23:** 57–62.

Alper, S., Duncan, L., and Losick, R. (1994) An adenosine nucleotide switch controlling the activity of a cell type-specific transcription factor in *B. subtilis*. *Cell* **77:** 195–205.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25:** 3389–3402.

Aravind, L., and Koonin, E.V. (2000) The STAS domain – a link between anion transporters and antisigma-factor antagonists. *Curr Biol* **10:** R53–R55.

Beaucher, J., Rodrigue, S., Jacques, P.E., Smith, I., Brzezinski, R., and Gaudreau, L. (2002) Novel *Mycobac-*

terium tuberculosis anti-sigma factor antagonists control sigmaF activity by distinct mechanisms. *Mol Microbiol* **45:** 1527–1540.

Chaturongakul, S., and Boor, K.J. (2004) RsbT and RsbV contribute to sigmaB-dependent survival under environmental, energy, and intracellular stress conditions in *Listeria monocytogenes*. *Appl Environ Microbiol* **70:** 5349–5356.

Chaturongakul, S., and Boor, K.J. (2006) SigmaB activation under environmental and energy stress conditions in *Listeria monocytogenes*. *Appl Environ Microbiol* **72:** 5197–5203.

Darnell, C.L., Hussa, E.A., and Visick, K.L. (2008) The putative hybrid sensor kinase SypF coordinates biofilm formation in *Vibrio fischeri* by acting upstream of two response regulators, SypG and VpsR. *J Bacteriol* **190:** 4941–4950.

Dufour, A., and Haldenwang, W.G. (1994) Interactions between a *Bacillus subtilis* anti-sigma factor (RsbW) and its antagonist (RsbV). *J Bacteriol* **176:** 1813–1820.

Dutta, R., and Inouye, M. (2000) GHKL, an emergent ATPase/kinase superfamily. *Trends Biochem Sci* **25:** 24–28.

Fouet, A., Namy, O., and Lambert, G. (2000) Characterization of the operon encoding the alternative sigma(B) factor from *Bacillus anthracis* and its role in virulence. *J Bacteriol* **182:** 5036–5045.

Hua, L., Hefty, P.S., Lee, Y.J., Lee, Y.M., Stephens, R.S., and Price, C.W. (2006) Core of the partner switching signalling mechanism is conserved in the obligate intracellular pathogen *Chlamydia trachomatis*. *Mol Microbiol* **59:** 623–636.

Hughes, K.T., and Mathee, K. (1998) The anti-sigma factors. *Annu Rev Microbiol* **52:** 231–286.

Hussa, E.A., Darnell, C.L., and Visick, K.L. (2008) RscS functions upstream of SypG to control the *syp* locus and biofilm formation in *Vibrio fischeri*. *J Bacteriol* **190:** 4576–4583.

Hussa, E.A., O'Shea, T.M., Darnell, C.L., Ruby, E.G., and Visick, K.L. (2007) Two-component response regulators of *Vibrio fischeri*: identification, mutagenesis, and characterization. *J Bacteriol* **189:** 5825–5838.

Kim, H.S., Park, S.J., and Lee, K.H. (2009) Role of NtrC-regulated exopolysaccharides in the biofilm formation and pathogenic interaction of *Vibrio vulnificus*. *Mol Microbiol* **74:** 436–453.

Knobloch, J.K., Jager, S., Horstkotte, M.A., Rohde, H., and Mack, D. (2004) RsbU-dependent regulation of *Staphylococcus epidermidis* biofilm formation is mediated via the alternative sigma factor sigmaB by repression of the negative regulator gene *icaR*. *Infect Immun* **72:** 3838–3848.

Kozak, N.A., Mattoo, S., Foreman-Wykert, A.K., Whitelegge, J.P., and Miller, J.F. (2005) Interactions between partner switcher orthologs BtrW and BtrV regulate type III secretion in *Bordetella*. *J Bacteriol* **187:** 5665–5676.

Magnin, T., Lord, M., and Yudkin, M.D. (1997) Contribution of partner switching and SpoIIAA cycling to regulation of sigmaF activity in sporulating *Bacillus subtilis*. *J Bacteriol* **179:** 3922–3927.

Manganelli, R., Provvedi, R., Rodrigue, S., Beaucher, J., Gaudreau, L., and Smith, I. (2004) Sigma factors and global gene regulation in *Mycobacterium tuberculosis*. *J Bacteriol* **186:** 895–902.

Mattoo, S., Foreman-Wykert, A.K., Cotter, P.A., and Miller, J.F. (2001) Mechanisms of *Bordetella pathogenesis*. *Front Biosci* **6:** E168–E186.

Mattoo, S., Yuk, M.H., Huang, L.L., and Miller, J.F. (2004) Regulation of type III secretion in *Bordetella*. *Mol Microbiol* **52:** 1201–1214.

Mittenhuber, G. (2002) A phylogenomic study of the general stress response sigma factor sigmaB of *Bacillus subtilis* and its regulatory proteins. *J Mol Microbiol Biotechnol* **4:** 427–452.

Miyazaki, E., Chen, J.M., Ko, C., and Bishai, W.R. (1999) The *Staphylococcus aureus rsbW* (orf159) gene encodes an anti-sigma factor of SigB. *J Bacteriol* **181:** 2846–2851.

Najafi, S.M., Willis, A.C., and Yudkin, M.D. (1995) Site of phosphorylation of SpoIIAA, the anti-anti-sigma factor for sporulation-specific sigma F of *Bacillus subtilis*. *J Bacteriol* **177:** 2912–2913.

Nyholm, S.V., and McFall-Ngai, M.J. (2003) Dominance of *Vibrio fischeri* in secreted mucus outside the light organ of *Euprymna scolopes*: the first site of symbiont specificity. *Appl Environ Microbiol* **69:** 3932–3937.

Nyholm, S.V., and McFall-Ngai, M.J. (2004) The winnowing: establishing the squid–*Vibrio* symbiosis. *Nat Rev Microbiol* **2:** 632–642.

Nyholm, S.V., Stabb, E.V., Ruby, E.G., and McFall-Ngai, M.J. (2000) Establishment of an animal–bacterial association: recruiting symbiotic *Vibrios* from the environment. *Proc Natl Acad Sci USA* **97:** 10231–10235.

Price, C.W., Fawcett, P., Ceremonie, H., Su, N., Murphy, C.K., and Youngman, P. (2001) Genome-wide analysis of the general stress response in *Bacillus subtilis*. *Mol Microbiol* **41:** 757–774.

Rantanen, M.K., Lehtio, L., Rajagopal, L., Rubens, C.E., and Goldman, A. (2007) Structure of *Streptococcus agalactiae* serine/threonine phosphatase. The subdomain conformation is coupled to the binding of a third metal ion. *FEBS J* **274:** 3128–3137.

van Schaik, W., Tempelaars, M.H., Zwietering, M.H., de Vos, W.M., and Abee, T. (2005) Analysis of the role of RsbV, RsbW, and RsbY in regulating {sigma}B activity in *Bacillus cereus*. *J Bacteriol* **187:** 5846–5851.

Shi, Y. (2009) Serine/threonine phosphatases: mechanism through structure. *Cell* **139:** 468–484.

Stabb, E.V. (2006) The *Vibrio fischeri–Euprymna scolopes* light organ symbiosis. In *The Biology of Vibrios*. Thompson, F.L., Austin, B., and Swings, J. (eds). Washington, DC, USA: ASM Press, pp. 204–218.

Stock, A.M., Robinson, V.L., and Goudreau, P.N. (2000) Two-component signal transduction. *Annu Rev Biochem* **69:** 183–215.

Visick, K.L. (2009) An intricate network of regulators controls biofilm formation and colonization by *Vibrio fischeri*. *Mol Microbiol* **74:** 782–789.

Visick, K.L., and Ruby, E.G. (2006) *Vibrio fischeri* and its host: it takes two to tango. *Curr Opin Microbiol* **9:** 632–638.

Visick, K.L., and Skoufos, L.M. (2001) Two-component sensor required for normal symbiotic colonization of *Euprymna scolopes* by *Vibrio fischeri*. *J Bacteriol* **183:** 835–842.

Voelker, U., Voelker, A., and Haldenwang, W.G. (1996) Reactivation of the *Bacillus subtilis* anti-sigma B antagonist,

RsbV, by stress- or starvation-induced phosphatase activities. *J Bacteriol* **178:** 5456–5463.

Yang, X., Kang, C.M., Brody, M.S., and Price, C.W. (1996) Opposing pairs of serine protein kinases and phosphatases transmit signals of environmental stress to activate a bacterial transcription factor. *Genes Dev* **10:** 2265–2275.

Yildiz, F.H., and Visick, K.L. (2009) *Vibrio* biofilms: so much the same yet so different. *Trends Microbiol* **17:** 109–118.

Yip, E.S., Grublesky, B.T., Hussa, E.A., and Visick, K.L. (2005) A novel, conserved cluster of genes promotes symbiotic colonization and sigma-dependent biofilm formation by *Vibrio fischeri*. *Mol Microbiol* **57:** 1485–1498.

Yip, E.S., Geszvain, K., DeLoney-Marino, C.R., and Visick, K.L. (2006) The symbiosis regulator *rscS* controls the *syp* gene locus, biofilm formation and symbiotic aggregation by *Vibrio fischeri*. *Mol Microbiol* **62:** 1586–1600.

Yuk, M.H., Harvill, E.T., Cotter, P.A., and Miller, J.F. (2000) Modulation of host immune responses, induction of apoptosis and inhibition of NF-kappaB activation by the *Bordetella* type III secretion system. *Mol Microbiol* **35:** 991–1004.