Singular structures and operon organizations of essential two-component systems in species of *Streptococcus*

Two component regulatory systems (TCSs) are found in all prokaryotes except mycoplasmas (Fraser *et al.*, 1995; Himmelreich *et al.*, 1996). They play critical roles in sensing and responding to environmental conditions and in bacterial pathogenesis (Hoch, 2000; Inouye & Dutta, 2003; Stock *et al.*, 2000). TCSs consist of a sensor histidine kinase protein,

which is usually bound to the bacterial membrane (Wolanin & Stock, 2003), and a cognate response regulator protein, which often acts as a DNA-binding transcription regulator. Autophosphorylation of the histidine kinase component in response to an environmental signal is followed by phosphoryl transfer to the cognate response regulator, which alters the expression of regulon genes needed to respond to the environmental condition.

Most TCSs are not required for bacteria to grow in the absence of stress in laboratory media (Kobayashi *et al.*, 2001; Oshima *et al.*, 2002). However, there are exceptions to this generalization in certain Gram-negative bacteria [e.g. *Caulobacter crescentus* (Quon *et al.*, 1996)] and in all Gram-positive bacteria with low G+C content in their DNA. Each of these

Gram-positive species, which include many important human pathogens (see Table 1), contains homologues of a single essential TCS, whose histidine kinase and response regulator are designated YycG and YycF, respectively. The gene encoding the YycF response regulator is required for growth of these Gram-positive bacteria under all conditions tested (Echenique & Trombe, 2001; Fabret & Hoch, 1998; Martin *et al.*, 1999; Ng *et al.*, 2003). The YycG histidine kinase is required for growth of most Gram-positive species tested to date (Fabret & Hoch, 1998; Martin *et al.*, 1999) or is conditionally required in *Streptococcus pneumoniae* depleted for the YycF response regulator (Ng *et al.*, 2003).

The genes regulated and signal(s) sensed by these essential TCSs in different

Table 1. Characteristics of YycG histidine kinases from Gram-positive bacteria with fully sequenced genomes

Species	YycG homologue*	Number of predicted TM domains†	Presence of YycH and YycI homologues‡	Capacity for electron transport§
<i>Streptococcus pneumoniae</i>	Spr1106, SP1226	1	No	No
<i>Streptococcus mitis</i>	Smt0611	1	No	No
<i>Streptococcus mutans</i>	Smu.1516	1	No	No
<i>Streptococcus agalactiae</i>	SAG0720, Gbs0742	1	No	Yes
<i>Streptococcus pyogenes</i>	Spy0529, SpyM18_0595, SpyM3_0373, SPs1480	1	No	No
<i>Enterococcus faecalis</i>	EF1194	2 (142)	Yes	Yes
<i>Lactobacillus plantarum</i>	Lp_0037	2 (147)	Yes	Yes
<i>Lactobacillus johnsonii</i>	LJ0066	2 (142)	Yes	Yes
<i>Lactococcus lactis</i>	L0124	2 (4)	No	Yes
<i>Listeria innocua</i>	Lin0316	2 (148)	Yes	Yes
<i>Listeria monocytogenes</i>	Lmo0288	2 (148)	Yes	Yes
<i>Bacillus cereus</i>	BC5462, BCE5614	2 (154)	Yes	Yes
<i>Bacillus anthracis</i>	BA5714	2 (154)	Yes	Yes
<i>Bacillus subtilis</i>	Bsu4037	2 (148)	Yes	Yes
<i>Staphylococcus epidermidis</i>	SE0019	2 (147)	Yes	Yes
<i>Staphylococcus aureus</i>	SA0018, SAV0019, MW0019	2 (145)	Yes	Yes
<i>Bacillus halodurans</i>	BH4026	2 (146)	Yes	Yes
<i>Oceanobacillus iheyensis</i>	OB3451	2 (154)	Yes	Yes

*YycG homologues were identified by BLAST searches of each complete genome sequence using *B. subtilis* *yycG* as the query. Only the match with highest homology in each genome was analysed. Multiple listings represent homologues in different strains of the same species.

†Number of transmembrane (TM) domains was predicted by the TMHMM program (Krogh *et al.*, 2001). A single TM domain, first noted in the YycG homologue of *S. pneumoniae* (Lange *et al.*, 1999) (see Fig. 1b), is present in the YycG homologues of all *Streptococcus* species (see text). Numbers in parentheses indicate the number of amino acid residues in predicted extracytoplasmic loops of YycG homologues with two TM domains (see Fig. 1a).

‡*B. subtilis* *yycH* and *yycI* were used as queries to search for homologues in each completed genome. Homologues of *B. subtilis* *yycI* are present in all genomes analysed (see text).

§Capacity for electron transport based on the presence of at least one homologue of the following *B. subtilis* genes encoding aerobic terminal oxidases (QoxABCD, CtaCDEF, CydAB and YthAB) (von Wachenfeldt & Hederstedt, 2002) or anaerobic nitrate reductase (NarGHII) (Hoffmann *et al.*, 1995).

Gram-positive species are of considerable interest. Recent results from several laboratories indicate that the regulons controlled by YycFG TCSs seem to be different in different Gram-positive bacteria (Dubrac & Msadek, 2004; Fukuchi *et al.*, 2000; Howell *et al.*, 2003; Ng *et al.*, 2003). However, the pattern emerging suggests that YycFG controls genes critical for cell wall biosynthesis, cell division and virulence (Dubrac & Msadek, 2004; Fukuchi *et al.*, 2000; Howell *et al.*, 2003; Ng *et al.*, 2003, 2004). The signal(s) sensed by the YycG histidine kinase homologues are not known (Claverys & Havarstein, 2002; Dubrac & Msadek, 2004; Fukuchi *et al.*, 2000; Howell *et al.*, 2003; Ng *et al.*, 2003). We describe here a potentially important structural difference in the YycG homologues and the operons that encode them for species of *Streptococcus* compared to those of other Gram-positive bacteria.

All YycG homologues contain a HAMP (linker) domain, a PAS domain, a histidine kinase domain (HisKin, which contains the phosphorylated histidine residue) and an ATPase domain (Fig. 1; Aravind & Ponting, 1999; Inouye & Dutta, 2003; Taylor & Zhulin, 1999). PAS domains of histidine kinases bind cofactors that respond to redox state (Taylor & Zhulin, 1999) and also act as sites of protein–protein interactions (Elsen *et al.*, 2003; Taylor & Zhulin, 1999; Wang *et al.*,

2001). Most histidine kinases, including YycG homologues from all Gram-positive species except for *Streptococcus*, are likely bound to the cytoplasmic membrane by two transmembrane domains that flank an extracytoplasmic loop (Fig. 1a). These extracytoplasmic loops are thought to play roles in sensing by some histidine kinases (Hoch, 2000; Wolanin & Stock, 2003). The extracytoplasmic loops of these YycG homologues are generally large and contain 142–154 amino acids (Table 1). In contrast, the YycG homologues of *Streptococcus* species contain a single predicted transmembrane domain (Fig. 1b), and hence lack extracytoplasmic loops (Table 1). The number of extracellular amino acids that protrude from these single transmembrane domains is small (4–12 amino acids; Fig. 1b; Table 1).

We noticed another structural difference between the YycFG TCS homologues of *Streptococcus* and those of other Gram-positive species. The genes encoding the YycFG two-component system are cotranscribed in all Gram-positive bacteria with another gene, designated *yycJ* (Fukuchi *et al.*, 2000; Wagner *et al.*, 2002). The YycJ protein contains a predicted HxHxDH metal-binding site in a β -lactamase fold (Daiyasu *et al.*, 2001). This type of metal-binding site binds zinc or iron ions in enzymes with different activities (Daiyasu *et al.*, 2001). The

function(s) of YycJ is unknown, although the YycJ homologue of *S. pneumoniae* is conditionally essential for growth in bacteria limited for expression of the YycF response regulator (Ng *et al.*, 2003). However, in Gram-positive species other than *Streptococcus*, *yycFG* and *yycJ* are cotranscribed with two additional genes, designated *yycH* and *yycI*, which encode proteins containing transmembrane domains (Fabret & Hoch, 1998; Fukuchi *et al.*, 2000). With one exception, Gram-positive species that encode YycH and YycI homologues contain YycG homologues with two transmembrane domains flanking extracytoplasmic loops (Fig. 1a; Table 1). The exception here is *Lactococcus lactis*, which encodes a YycG homologue with two transmembrane domains, but lacks *yycH* and *yycI* genes (Table 1). However, the *L. lactis* YycG homologue is itself unusual in that it contains a predicted extracytoplasmic loop of just 4 amino acids, which is much smaller than those of other YycG homologues (Fig. 1a).

The structural and operon differences of the YycFG TCSs of *Streptococcus* species compared to those of other Gram-positive species suggest important differences in signal transduction. There are clearly two distinct classes of YycG histidine kinases (Fig. 1; Table 1), which tends to argue against the view that the extracytoplasmic domains of YycG homologues became randomized during evolution (Hoch, 2000). Moreover, a different subset of associated components (YycJ, YycH and YycI) is associated with each YycG class (Table 1). These structural differences imply that different signals or subsets of signals may be sensed by YycG homologues in *Streptococcus* species compared to those sensed in other Gram-positive species. It is noteworthy that Gram-positive species containing YycG histidine kinases with two transmembrane domains flanking large extracytoplasmic loops (Fig. 1a) and associated YycH and YycI components have the capacity for electron transport, which is absent from all species of *Streptococcus*, except for *Streptococcus agalactiae* (Table 1). Perhaps, the extended extracytoplasmic domain of YycG and the YycH and YycI components sense the redox state of electron transport.

Fig. 1. Structural motifs in YycG histidine kinase homologues. See text for details.

On the other hand, the cytoplasmic PAS domains of all YycG homologues and the associated YycJ components may sense small molecule and cofactor signals that reflect metabolic state, such as those that accumulate during aerobic or anaerobic growth or stress (Nakano & Zuber, 1998; Taylor & Zhulin, 1999). The metabolisms and physiological niches of different Gram-positive bacteria vary considerably. These differences may be reflected by the different sets of genes regulated by the YycFG TCSs (Dubrac & Msadek, 2004; Fukuchi *et al.*, 2000; Howell *et al.*, 2003; Ng *et al.*, 2003). It remains to be determined whether the same or different small molecules' signals are sensed by YycG homologues in these disparate species.

Acknowledgements

Supported by research funds from Indiana University Bloomington. Wai-Leung Ng is a predoctoral trainee on grant NIGM-T32GM0775 from the National Institutes of Health of the USA.

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DOI 10.1099/mic.0.27550-0