Quorum Sensing in Competence and Sporulation

Navneet Rai, Rewa Rai, and K.V. Venkatesh

Introduction

In several Gram-positive bacteria, competence and sporulation are few of several physiological processes controlled by quorum sensing (QS). Competence is a phenomenon wherein a bacterium acquires extracellular DNA for its maintenance. Cells committed for competence halt DNA replication and do not form stable RNA. Only a fraction of cells (10–20 %), in a population, develop competence, at a particular window of growth phase, and in response upregulate expression of genes involved in the uptake and processing of extracellular DNA (Maamar and Dubnau 2005). Sporulation, second QS-controlled phenotype, occurs under extreme stress and nutritional scarcity. Prolonged nutrient deprivation compels the cell to enter the process of sporulation, the outcome of which is the production of a metabolically dormant endospore that resumes growth once the conditions become favorable again. Endospores can sustain high temperature, pressure, and anhydrous conditions. Spore formation is a complex and tightly regulated phenomenon, where several hundred genes are directly and indirectly involved (Narula et al. 2012). Some examples of well-studied Gram-positive bacteria, where QS regulates competence and sporulation, are *Streptococcus species*, *Bacillus subtilis*, and sporulation in *B. subtilis* (Lazazzera et al. 1997).

Autoinducer peptides (AIPs) are the QS molecules, which regulate competence and sporulation. AIPs are posttranslationally modified oligopeptides, produced and recognized by a diverse range of Gram-positive bacteria. Some AIPs freely diffuse across the cell membrane, and some are actively transported. These signaling molecules are recognized by a membrane-bound histidine kinase receptor. Reception signaling initiates a phosphorylation cascade that alters the activity of a DNA binding transcriptional regulatory protein termed as the response regulator. Each Gram-positive bacterium responds to a specific type of signaling peptide by using a specific type of membrane-bound receptor (Waters and Bassler 2005).

Competence in *Streptococcus pneumoniae*

S. pneumoniae infection causes pneumonia and sepsis. During the last couple of decades, this bacterium has acquired resistance against several antibiotics. One of the many reasons responsible for the development of antibiotic resistance is the horizontal transfer of antibiotic resistance genes

N. Rai (✉)
Genome Center, University of California Davis, Davis, CA, USA
e-mail: nrai.iitb@gmail.com

R. Rai
Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, India

K.V. Venkatesh
Department of Chemical Engineering, Indian Institute of Technology Bombay, Powai, Mumbai, India

V.C. Kalia (ed.), *Quorum Sensing vs Quorum Quenching: A Battle with No End in Sight*,
DOI 10.1007/978-81-322-1982-8_6, © Springer India 2015

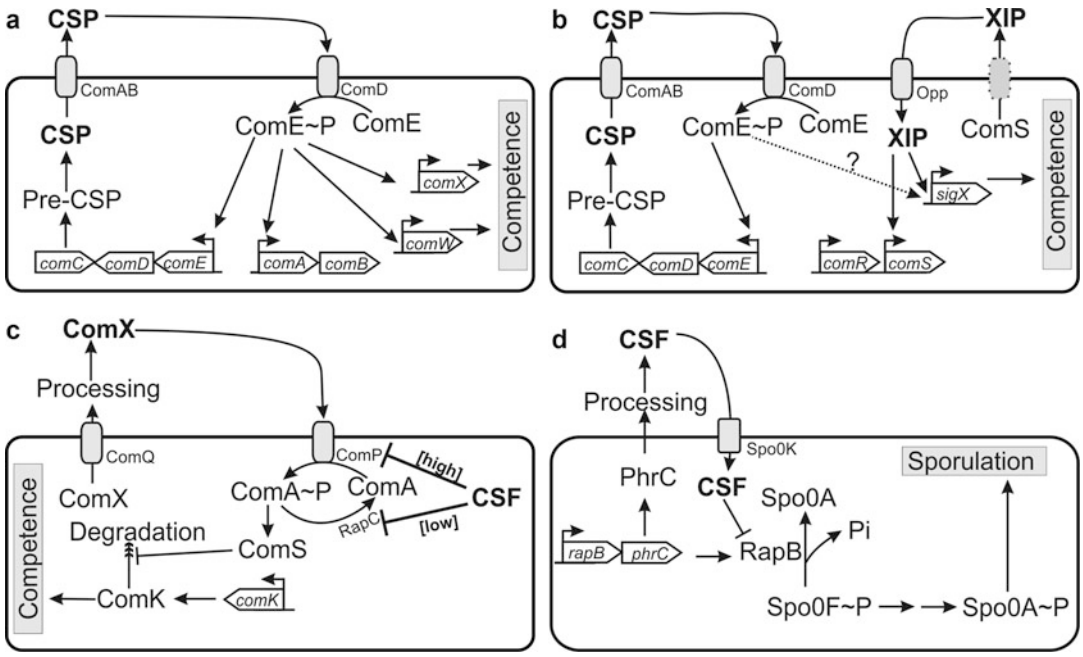

Fig. 1 Quorum-sensing-regulated competence and sporulation pathways. (**a**) CSP-mediated competence development in *S. pneumoniae*. (**b**) CSP- and XIP-mediated competence development in *S. mutans*. (**c**) ComX- and CSF-mediated competence development in *B. subtilis*. (**d**) CSF-mediated sporulation in *B. subtilis*

among bacteria. Bacteria susceptible to antibiotic get transformed into antibiotic resistance form during their competent phase by acquiring foreign antibiotic resistance gene(s). *S. pneumoniae* develops competence during mid-log phase of growth. QS signaling molecule, competence using competence and sporulating peptide (CSP), drives the competence development in *S. pneumoniae* (Fig. 1a) (Weng et al. 2013). CSP is the product of *comC* gene, which once synthesized, is modified, and transported out by the membrane-bound transporter, ComAB. Extracellular concentration of CSP increases as the cell density increases. Once cell density reaches a threshold value (10^7 cells/ml, corresponds to 1–10 ng/ml CSP), CSP binds to a membrane-bound histidine kinase, ComD. CSP bound ComD gets autophosphorylated and subsequently transfers the phosphate group to a response regulator protein, ComE. Phosphorylated ComE activates early and late competence genes, directly and indirectly (Johnsborg and Havarstein 2009). It has been observed that expression of early competence genes is maximum during 6–7 min and late genes during 9–10 min, after activation of this pathway (Peterson et al. 2000). ComE ~ P activates early competence genes, including *comAB* and *comCDE*. ComE is the product of *comE* of *comCDE* operon, and hence, by activating *comCDE*, ComE positively regulates its own expression, creating a sensitive autocatalytic loop. ComE ~ P activates late competence genes indirectly by upregulating transcriptions of *comX* and *comW*, which in turn upregulate late competence genes involved in the binding, uptake, and recombination of the extracellular DNA (Luo et al. 2004).

Competence in *Streptococcus mutans*

S. mutans forms biofilms on dental surfaces, preventing the removal of bacteria, consequently, leading to the decay of teeth. In *S. mutans*, QS regulates competence using two pathways, ComRS and ComCDE (Fig. 1b). ComRS pathway was discovered recently and is the major QS signaling system involved in the development

of competence in *S. mutans*. In ComRS regulatory network, *comS* encodes an immature AIP, ComS, which is transported and processed by the unknown mechanism and converted in to mature hydrophobic AIP, XIP (*sigX i*nducing *p*eptide). XIP is internalized via Opp/Ami class of ABC transporter. Once above the threshold concentration inside the cell, XIP binds with an Rgg family transcription factor, ComR, and forms a complex. XIP-ComR complex activates *comS*, early, and late competence genes by upregulating the transcription of *sigX* (also called *comX*). Concentration of SigX is maximum during transition from late log phase to stationary phase, indicating that cells are more competent during this phase. Second competence regulatory machinery in *S. mutans*, ComCDE, is similar to the ComCDE competence development machinery of *S. pneumoniae*. But in *S. mutans*, it is not clear, how does ComE regulate the expression of late competence genes. It has been hypothesized that at high cell density, ComE upregulates the expression of *sigX*, directly or indirectly, which in turn, activates competence genes (Mashburn-Warren et al. 2010).

Competence and Sporulation in *Bacillus subtilis*

B. subtilis is a soil bacterium, uses peptide quorum-sensing signal molecules to choose one of two physiological stages, competence and sporulation, depending on environmental conditions and growth phase. *B. subtilis* develops competence during transition between logarithmic and stationary phases of growth. Two QS peptides, ComX and CSF (competence and sporulation factor), facilitate competence development in *B. subtilis*. ComX is derived from pre-ComX, a 55 amino acids long peptide. Mature ComX is 10 amino acids long peptide with an isoprenyl modification at a tryptophan residue (Okada et al. 2005). ComQ is required for the posttranslational modification of ComX and its secretion into the extracellular medium (Bacon Schneider et al. 2002). Extracellular ComX is detected by the membrane-bound histidine sensor kinase, ComP. Once above threshold concentration, ComX binds to ComP and stimulates its autophosphorylation, which later transfers phosphate to the DNA-binding response regulator ComA. ComA $\sim$ P induces the expression of *comS*. ComS plays a significant role in maintaining the high concentration of the competence master regulator protein, ComK, by preventing its targeted degradation in the cell (Fig. 1c). High level of ComK produced in the cell, regulates the late competence operons, leading to the synthesis of proteins required for the DNA uptake, processing, and integration into the genome. Late competence operons include *comG, comE, comF,* and *comC*. The DNA uptake system of *B. subtilis* does not show any specificity for the foreign DNA sequences or the origin of the foreign DNA and is internalized with same specificity, as a single-stranded DNA, which is further recombined with the host genome. A nuclease located in the cell membrane, encoded by *nucA*, cleaves the DNA resulting in a fragment of about 20 kb. The complementary strand is degraded, with the release of nucleotides into the medium and the single-stranded DNA is taken up into the cell. After internalization of the single-stranded DNA, it is associated with bacterial recombination proteins, such as RecA and AddAB, which integrates the single-stranded DNA into the host genome (Hamoen et al. 2003).

Second QS molecule CSF is encoded by *phrC*. CSF is a pentapeptide derived from the C-terminal end of a 40 amino acid long secreted polypeptide, PhrC. CSF is released in the extracellular medium by the simple diffusion. With the increase in cell density the extracellular level of CSF increases. CSF is transported back into the cell by the Opp peptide transporter and counteracts the action of RapC protein, subsequently, leading to the synthesis of ComS by ComA $\sim$ P (Fig. 1d). Presence of ComS stops targeted degradation of ComK, hence activates competence. CSF inhibits RapC phosphatase when present at a relatively lower concentration of 1–10 nM. But, at a relatively higher concentration of >20 nM, CSF binds to and inhibits the phosphorylation of ComP, hence, decreasing the level of active ComA $\sim$ P in the

cell, which subsequently leads to an inhibition of ComS synthesis, resulting in the inhibition of competence development. Also, at such higher concentration, CSF inhibits the activity of an aspartyl-phosphate phosphatase, RapB. RapB dephosphorylates sporulation response regulator protein, Spo0F ~ P. Inhibition of RapB results in the accumulation of high levels of Spo0F ~ P which further drives the synthesis of high levels of Spo0A ~ P in the cell. The presence of high level of Spo0A ~ P favors the establishment of sporulation in the cell. Hence, at higher concentrations, CSF compels the cell to progress toward the sporulation pathway by two paths, inhibition of continued synthesis of ComK that is switching off the synthesis of competence machinery and activation of components of the sporulation phosphorelay (Lopez and Kolter 2010).

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