Biotechnology Advances 35 (2017) 505–511

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Is there any crosstalk between the chemotaxis and virulence induction signaling in *Agrobacterium tumefaciens*?

Minliang Guo *, Zhiwei Huang, Jing Yang

College of Bioscience and Biotechnology, Yangzhou University, Yangzhou, Jiangsu 225009, China

ARTICLE INFO

Article history:
Received 4 September 2016
Received in revised form 17 March 2017
Accepted 22 March 2017
Available online 23 March 2017

Keywords:
Chemotaxis
Agrobacterium tumefaciens
Virulence induction
Signal transduction
Chemoreceptor
Histidine autokinase
Response regulator
Two-component system
Signaling crosstalk

ABSTRACT

Agrobacterium tumefaciens, a soil-born phytopathogenic bacterium, is well known as a nature's engineer due to its ability to genetically transform the host by transferring a DNA fragment (called T-DNA) from its Ti plasmid to host-cell genome. To combat the harsh soil environment and seek the appropriate host, *A. tumefaciens* can sense and be attracted by a large number of chemical compounds released by wounded host. As a member of α-proteobacterium, *A. tumefaciens* has a chemotaxis system different from that found in *Escherichia coli*, since many chemoattractants for *A. tumefaciens* chemotaxis are virulence (*vir*) inducers. However, advances in the study of the chemotaxis paradigm, *E. coli* chemotaxis system, have provided enough information to analyze the *A. tumefaciens* chemotaxis. At low concentration, chemoattractants elicit *A. tumefaciens* chemotaxis and attract the species to the wound sites of the host. At high concentration, chemoattractants induce the expression of virulence genes and trigger T-DNA transfer. Recent studies on the VirA and ChvE of the *vir*-induction system provide some evidences to support the crosstalk between chemotaxis and *vir*-induction. This review compares the core components of chemotaxis signaling system of *A. tumefaciens* with those observed in other species, discusses the connection between chemotaxis and *vir*-induction in *A. tumefaciens*, and proposes a model depicting the signaling crosstalk between chemotaxis and *vir*-induction.

Contents

1. Introduction

Motile bacteria and archaea are able to track the gradients of attractants and repellents in their surroundings and to move towards more favorable living environments. This behavior is called chemotaxis (Adler, 1966; Wadhams and Armitage, 2004). Chemotactic behavior is essential not only for bacteria to survive under nutrient stress, but also for pathogenic bacteria to invade their hosts (Erhardt, 2016; Falke and Piasta, 2014; Sourjik and Wingreen, 2012). Chemotaxis significantly affects the development of bacterial biofilm (Alexandre, 2015; He and Bauer, 2014; Mangwani et al., 2016; Merritt et al., 2007) and the establishment of symbiotic associations of bacteria with plants (Scharf et al., 2016). In addition, chemotaxis enhances bacteria to be attracted to

* Corresponding author.
E-mail address: guoml@yzu.edu.cn (M. Guo).

http://dx.doi.org/10.1016/j.biotechadv.2017.03.008
0734-9750/

biodegradable pollutants and thus increases the bioavailability and biodegradation rate of the pollutants (Adadevoh et al., 2016; Krell et al., 2013; Kudryasheva and Tarasova, 2015; Mangwani et al., 2016; Montaño et al., 2013). *Agrobacterium tumefaciens* is a species of the genus *Agrobacterium*, a group of Gram-negative soil bacteria often associated with plants. Most species of this genus cause disease on plants, and *A. tumefaciens* causes the crown gall disease in various dicotyledonous plants, which, as a motile α-proteobacterium, possesses a highly sensitive chemotaxis system to sense and be attracted to a broad range of sugars, amino acids, and phenolic compounds (McCullen and Binns, 2006; Shaw, 1991; Winans, 1992). For motile, *A. tumefaciens* produces a small tuft of up to 6 flagella that are typically localized at or around a single pole of the cell (Chesnokova et al., 1997). Only the flagella-mediated swimming motility is recognized to occur in *A. tumefaciens* and there is no evidence of alternate motility mechanisms occurring in this bacterium (Mohari et al., 2015). The flagella in *A. tumefaciens* rotate clockwise to propel the bacterial cells forward. In contrast to the *E. coli* paradigm, tumbling in *A. tumefaciens* is thought to occur due to asynchronous slowing of flagellar rotation, resulting in disruption of the flagellar bundle, rather than a reversal of flagellar rotation (Merritt et al., 2007; Tomlinson and Fuqua, 2009).

A. tumefaciens induces crown gall tumor in plants by transferring and integrating a segment of its Ti plasmid DNA into the host-plant genome (Guo et al., 2011; Matveeva and Lutova, 2014; Nester, 2015). Major steps of the genetic transformation process comprise: 1) sensing and chemotaxis of *Agrobacterium* to the wound site of host-plant, 2) induction and expression of virulence genes of *Agrobacterium*, 3) generation and processing of the transferred DNA (T-DNA), 4) transportation of T-DNA and virulence proteins into host cells by the type IV secretory system (T4SS), and 5) integration and expression of T-DNA in host cells. The extraordinary pathogenesis of *A. tumefaciens* has made it serve as a model system for the studies of pathogen-host signal exchange, bacterial T4SS and interkingdom macromolecular transfer (Guo et al., 2009, 2011; Pitzschke, 2013). Within the past two decades, most studies on *A. tumefaciens* were focused on the molecular mechanisms of virulence induction (Lacroix and Citovsky, 2013; Yang et al., 2015), T-DNA transfer (Gao et al., 2013; Guo et al., 2007a; Nester, 2015), T4SS transporter (Chandran, 2013; Guo et al., 2007b; Low et al., 2014; Sakalis et al., 2014; Xu et al., 2016), and quorum sensing (Lang and Faure, 2014; Subramoni et al., 2014). Our understanding of the chemotaxis signaling mechanism of *A. tumefaciens* is far more limited. However, chemotaxis signal transduction system (Che) is very important for *A. tumefaciens* to recognize the host and to seek the wound site of the host plant in the bulk soil (Merritt et al., 2007; Tomlinson and Fuqua, 2009). Encouragingly, the studies on chemotaxis signaling systems in the model bacterium *E. coli* (Bi and Lai, 2015; Falke and Piasta, 2014; Jones and Armitage, 2015; Parkinson et al., 2015; Sourjik and Wingreen, 2012) and a few other bacterial species (Rao et al., 2008; Sampedro et al., 2015; Walukiewicz et al., 2014; Zautner et al., 2012) have made great advances. Therefore, in this review, we will firstly summarize the prototypical chemotaxis signaling system in *E. coli*, then compare agrobacterial chemotaxis signaling system with others, and finally discuss potential crosstalk between chemotaxis signaling system and virulence induction in *A. tumefaciens*.

2. Prototype of chemotaxis signaling system

The best-studied chemotaxis signaling system is that of *E. coli*, which was initiated almost half century ago (Adler, 1969). The relatively few components and simplicity of the *E. coli* chemotaxis system make it an attractive paradigm for chemotaxis studies (Hamer et al., 2010; Jones and Armitage, 2015; Parkinson et al., 2015). In the past decades, intensive studies on the *E. coli* Che system led to a full molecular understanding of how chemotaxis mediates *E. coli* cells to navigate in the gradients of various chemicals (Hazelbauer, 2012; Parkinson et al., 2015). Comparative genomics-based studies indicated the conservation of chemotaxis signaling principles in the *E. coli* Che system with six essential components: CheA, CheB, CheR, CheW, CheY and CheZ; and five transmembrane chemoreceptors (called methyl-accepting chemotaxis proteins, MCPs): Tar, Tsr, Tap, Trg and Aer (Baker et al., 2006; Porter et al., 2011; Wadhams and Armitage, 2004).

MCPs sense chemoeffectors (chemoattractants and chemorepellents) and transduce sensory signal to the interacting cytoplasmic proteins (Briegel et al., 2014). For example, Tsr from *E. coli* is the chemoreceptor for serine and Tar senses the attractants maltose and aspartate and the repellents Co^{2+} and Ni^{2+}. MCP molecules are homodimers. Three homodimers form a trimer of dimers. Thousands of trimers-of-dimers are packed into much larger macromolecular clusters in roughly hexagonal arrays. The dominating secondary structure in the MCP subunit is α-helix. MCP molecule can be divided into three functional elements: 1) a transmembrane sensing module including a periplasmic ligand-binding domain and four membrane-spanning helices, 2) a signal-conversion HAMP (histidine kinases, adenyl cyclases, MCPs and some phosphatases) domain mediating signaling transactions between transmembrane and cytoplasmic signaling regions, and 3) a kinase control module comprising the regions for adaptational modification and for docking and regulating CheA kinase (Briegel et al., 2012, 2014; Hazelbauer and Lai, 2010). CheA is a histidine autokinase (Bilwes et al., 1999; Miller et al., 2006). CheW is a scaffolding protein that couples dimeric CheA to the cytoplasmic kinase control domain of MCP (Cardozo et al., 2010). The kinase activity of dimeric CheA is regulated by MCP. One subunit of dimeric CheA uses ATP to transphosphorylate the other. Phosphorylated CheA can transfer the phosphoryl group to one of two response regulator proteins, CheY and CheB. Phosphorylated CheY is released from CheA and diffuses to the flagellar motor switch, where it binds to the flagellar motor proteins FliM and FliN, shifting the rotational direction of flagella from the default counter-clockwise (CCW) to clockwise (CW) (Saragosti et al., 2011; Sarkar et al., 2010). The change of rotational direction leads to tumbling, allowing the bacterium to reorient the cell body in a new swimming direction. Increase in attractant concentration or reduction in repellent concentration decreases the ability of MCP to activate CheA auto-phosphorylation, slowing the transfer of CheA phosphoryl groups to CheY or CheB. Low level of phosphorylated CheY lets bacterium to keep smooth swimming. CheZ is a phosphorylated CheY-specific phosphatase that can remove the phosphoryl group from the phosphorylated CheY to maintain a low level of phosphorylated CheY. CheR is a constitutively active MCP-specific methyltransferase that can transfer methyl groups to the glutamyl residues in the kinase control module of MCP (Springer and Koshland, 1977). Methylation of MCPs increases their ability to activate CheA, which in turn increases the phosphorylation of CheY and CheB. CheB is a methylesterase that can remove methyl groups from the MCPs. The methylesterase activity of CheB is activated by the phosphorylation catalyzed by CheA. Therefore, the phosphorylation of CheB reduces the methylation state of the MCPs, decreasing their ability to activate CheA. CheR and CheB work in concert to maintain the CheA activity by regulating the methylation state of the MCPs, resulting in adaptation. The methylation regulation of the MCPs is kinetically slower than the phosphorylation of CheY, resulting in a short time delay between adaptation and signaling excitation. This functions like a primitive memory (Krembel et al., 2015; Min et al., 2012). The chemotactic signaling pathway in *E. coli* is shown in Fig. 1. For more complex chemotaxis signaling pathways of the *E coli* chemotaxis system, interested readers may consult other recent reviews (Jones and Armitage, 2015; Micali and Endres, 2016; Parkinson et al., 2015).

3. Chemotaxis system in *A. tumefaciens*

3.1. Constituent of *A. tumefaciens* chemotaxis system

Although chemotaxis signaling principles identified in *E. coli* are conserved with most archaeal and bacterial species, many motile bacteria