Cell-cell communication in the plant pathogen *Agrobacterium tumefaciens*

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The plant pathogen *Agrobacterium tumefaciens* induces the formation of crown gall tumours at wound sites on host plants by directly transforming plant cells. This disease strategy benefits the bacteria as the infected plant tissue produces novel nutrients, called opines, that the colonizing bacteria can use as nutrients. Almost all of the genes that are required for virulence, and all of the opine uptake and utilization genes, are carried on large tumour-inducing (Ti) plasmids. The observation more than 25 years ago that specific opines are required for Ti plasmid conjugal transfer led to the discovery of a cell-cell signalling system on these plasmids that is similar to the LuxR–LuxI system first described in *Vibrio fischeri*. All Ti plasmids that have been described to date carry a functional LuxI-type *N*-acylhomoserine lactone synthase (TraI), and a LuxR-type signal receptor and transcriptional regulator called TraR. The *traR* genes are expressed only in the presence of specific opines called conjugal opines. The TraR–TraI system provides an important model for LuxR–LuxI-type systems, especially those found in the agriculturally important Rhizobiaceae family. In this review, we discuss current advances in the biochemistry and structural biology of the TraR–TraI system.

Keywords: *Agrobacterium tumefaciens*; tumour-inducing plasmids; signal receptor; conjugal opines

1. INTRODUCTION

A general interest in the soil bacterium *Agrobacterium tumefaciens* began with the observation, published in a series of papers almost 30 years ago, that wild-type strains of this organism have the unique ability to directly transform plant cells by transfer of discrete DNA fragments (Escobar & Dandekar 2003). This transformation causes the infected plant cells to overproduce phytohormones, causing cell proliferation, which results in the growth of tumours called crown galls. The transforming DNA (T-DNA) also encodes genes for the production of novel compounds called opines, which are sources of nutrients for the colonizing bacteria. By inducing plant cell growth and directing these cells to produce nutrients that only the colonizing bacteria can use, *A. tumefaciens* makes a novel niche for itself in its environment, giving itself a clear advantage over other plant-colonizing bacteria. Since these early discoveries, the possible utility of *A. tumefaciens* in plant genetic engineering certainly did not go unnoticed, and in fact plant transformation using *A. tumefaciens* is currently the most efficient method available for many types of plants (Gelvin 2003).

Almost all of the genes required for tumorigenesis are carried on large (approx. 200 kb) plasmids in pathogenic isolates of *A. tumefaciens* (Kerr 1969, 1971; Van Larebeke *et al.* 1974). These plasmids, called tumour-inducing (Ti) plasmids, have complete conjugation systems, yet conjugate only in the presence of specific opines (Genetello *et al.* 1977; Kerr *et al.* 1977). Therefore, Ti plasmid conjugation can only occur in the crown gall

tumour environment, as these tumours are the only natural sources of these compounds. The type of opine required for conjugation depended on the type of Ti plasmid (Genetello *et al.* 1977; Kerr *et al.* 1977).

Since these discoveries, it has been shown that the Ti plasmids also carry a quorum-sensing system related to the LuxR–LuxI system of *Vibrio fischeri* (Engbrecht *et al.* 1983; Engbrecht & Silverman 1984, 1987). The basic components of the system include the LuxI-type protein TraI, which synthesizes *N*-3-oxooctanoyl-homoserine lactone (3-oxo-C8 HSL; Fuqua & Winans 1994; Hwang *et al.* 1994; More *et al.* 1996), and the receptor of 3-oxo-C8 HSL, the LuxR-type protein TraR (Piper *et al.* 1993; Zhang *et al.* 1993; Fuqua & Winans 1994). All known LuxI proteins synthesize an *N*-acylhomoserine lactone (AHL), which is detected by the cognate LuxR homologue. At high population densities, 3-oxo-C8 HSL accumulates to some threshold level, and TraR-3-oxo-C8 HSL complexes then activate transcription of target genes on the Ti plasmid, including those required for Ti plasmid conjugation (Fuqua & Winans 1996a). Therefore, inducing concentrations of 3-oxo-C8 HSL are required for conjugation. It was later shown that the expression of *traR* genes requires specific opines called conjugal opines (Beck von Bodman *et al.* 1992; Fuqua & Winans 1996b). This explained the previous observation that conjugation requires opines.

Since the initial discovery of TraR and TraI, a series of papers have been published on quorum sensing in *A. tumefaciens*, including many of the first biochemical and structural studies of LuxR–LuxI-type systems. The regulation of TraR activity is rather complex, and we certainly do not have a complete picture of how this system works. However, our current model and available data do prompt many exciting questions about how this

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system works. In this review, we first present the background information relevant to quorum sensing in *A. tumefaciens* and then focus on our current knowledge of the molecular biology of the TraR–TraI system.

2. AGROBACTERIUM TUMEFACIENS, THE TUMOUR INDUCING PLASMIDS AND CROWN GALL DISEASE

Agrobacterium tumefaciens is a member of the Rhizobiaceae, which is in the α -subgroup of the proteobacteria. All members of this family are soil bacteria, and are traditionally divided into two groups. The rhizobia, which include members of the genera *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Azorhizobium* and *Bradyrhizobium*, are all nitrogen-fixing symbionts of host legumes. The agrobacteria include the plant pathogens *A. tumefaciens*, *Agrobacterium rubi*, *Agrobacterium vitis* and *Agrobacterium rhizogenes*, as well as the non-pathogenic *Agrobacterium radiobacter*. *Agrobacterium tumefaciens*, *A. rubi* and *A. vitis* all induce the formation of crown galls through plant transformation as described previously (Binns & Costantino 1998). In addition, *A. vitis*, which is restricted to grape, causes tumours on the crown of grape vines as well as necrotic lesions on grape roots (Burr & Otten 1999). *Agrobacterium rhizogenes* also causes disease by transforming plant cells, but induces the formation of ‘hairy roots’ rather than galls (Binns & Costantino 1998). Recently, it was proposed that the genus *Agrobacterium* be abandoned and that all species be placed in the genus *Rhizobium* (Young *et al.* 2003).

For the rhizobia and agrobacteria, intimate associations with host plants can be achieved only if the bacteria carry large self-transmissible genetic elements, either plasmids or integrating conjugative elements that include almost all of the determinants for either symbiosis or disease. Tumorigenic strains of *Agrobacterium* contain either Ti or root inducing plasmids. Functional LuxR–LuxI-type systems have been described for a number of the rhizobial symbiosis plasmids (Wisniewski-Dye & Downie 2002; Gonzalez & Marketon 2003). However, the TraR–TraI system of the Ti plasmids remains the only well-studied quorum-sensing system of this agriculturally important family.

The complete genome sequence of *A. tumefaciens* strain C58 has been published by two groups (Goodner *et al.* 2001; Wood *et al.* 2001). The genome is unusual in that it has both a circular and a linear chromosome. These two replicons carry all of the essential and housekeeping genes of the organism, although the linear chromosome has fewer of these per megabase than the circular chromosome. This and other evidence suggest that the linear chromosome may have actually evolved from a plasmid (Goodner *et al.* 2001; Wood *et al.* 2001). C58 also carries two large circular plasmids, pAtC58 (also called the ‘cryptic’ plasmid) and the Ti plasmid pTiC58. The *traR* and *traI* genes of the quorum-sensing system are both located on pTiC58, while a few additional *luxR* homologues are found in the genome sequence of C58. It is not yet clear whether these are functional as AHL receptors or not. Curiously, there are no corresponding *luxI*-type genes found anywhere on the genome.

Ti plasmids from different isolates of *A. tumefaciens* are traditionally classified by opine type. However, a variety of different opine synthases and their corresponding catabolism genes are encoded by each isolate (Zhu *et al.* 2000). In addition, there is often an overlap in the types of opines associated with different plasmids. The best-studied are the nopaline-type (including pTi37 and pTiC58) and the octopine-type (including A6, B6, Ach5, 15966 and R10) Ti plasmids. However, these octopine-type Ti plasmids are all virtually identical and a composite DNA sequence of these plasmids has been published (Zhu *et al.* 2000). The genes required for virulence (the *vir* genes), conjugation (*tra* and *trb* genes) and vegetative replication (*rep*) are highly conserved between the octopine-type and the nopaline-type Ti plasmids.

The *vir* genes are arranged in a number of operons, most of which are essential for plant cell transformation. The expression of these genes is induced only in response to signals released from plant wounds (phenolics, sugars and a pH range of 5.0–5.5; Johnson & Das 1998). These signals are detected by VirA, a transmembrane sensor kinase, which then phosphorylates its cognate response regulator, VirG (Stachel & Zambryski 1986; Winans *et al.* 1988). Phospho-VirG then activates the *vir* genes, which direct T-DNA processing from the Ti plasmid and its transfer into plant host cells (Gelvin 2003). In the host cell cytoplasm, the single-stranded T-DNA is targeted to the nucleus and integrated into host genomic DNA. T-DNA transfer requires a direct contact between the bacterium and the plant cell, and sequence analysis shows that the transfer apparatus has evolved from a bacterial conjugation system (Lessl *et al.* 1992; Kado 1994; Lessl & Lanka 1994; Pohlman *et al.* 1994). However, the *vir* system and the *tra* and *trb* genes required for Ti plasmid conjugation are not closely related. T-DNA transfer, which is an example of a type IV secretion system, and T-DNA integration into plant genomic DNA have been the subjects of intense study, but are beyond the scope of this review. We refer the reader to recent reviews of these fields (Escobar & Dandekar 2003; Gelvin 2003).

As alluded to previously, T-DNA-encoded genes expressed in the plant host cell direct phytohormone overproduction and opine synthesis (Morris 1990; Dessaux *et al.* 1992). The opines are taken up by the agrobacteria via dedicated ABC-type uptake systems, and used as sources of energy, carbon and, in some cases, nitrogen or phosphorus (Dessaux *et al.* 1998). The expression of opine uptake and catabolic genes is positively regulated by the cognate opines (Dessaux *et al.* 1992).

3. A MODEL OF QUORUM SENSING IN *A. TUMEFACIENS*

The regulation of TraR activity is quite complex and occurs at the levels of transcription, protein folding, resistance to proteolysis and the formation of quaternary structures with other TraR subunits and with other proteins. In this section, we will provide an overview of how the system works and the many factors involved in regulation. The model presented in figure 1 is a

Figure 2. A comparison of *traR* regulation via opines on different types of Ti plasmids. On octopine-type Ti plasmids, *traR* is activated by OccR in response to octopine. On nopaline-type Ti plasmids, *traR* is expressed when AccR repression is relieved by agrocinopines A and B. Regulation of *traR* on the chrysopine-type plasmid is similar, except that the inducing opines are agrocinopines C and D. There are two copies of *traR* on pAtK84b, one thought to be activated by NocR in response to nopaline and the transcription of the other is activated in response to agrocinopines A and B via the derepression of AccR.

pTiBo542, although in this case the mechanism of regulation is not known (Ellis *et al.* 1982). The non-pathogenic strain *A. radiobacter* K84 carries a plasmid called pATK84b, which does not carry *vir* genes or T-DNA, but does contain genes for opine uptake and utilization (Hayman & Farrand 1988, 1990; Clare *et al.* 1990). This allows *A. radiobacter* to compete with pathogenic strains for nutrients in or near crown galls. Conjugal transfer of pATK84b is regulated by two different *traR* genes (Oger & Farrand 2002). One copy, *traR_{noc}*, is in the *nox* operon, which is induced by nopaline, while *traR_{acc}* expression is induced by agrocinopines A and B.

(b) Post-transcriptional control of *TraR* activity

A fascinating form of post-transcriptional regulation of protein activity that is gaining increasing interest is the requirement of inducing ligands for protein stability. An increasing number of proteins that are involved in highly time-dependent processes (including transcription regulators) and that require their cognate ligands for protein folding have been discovered in both bacteria and eukaryotes (Dyson & Wright 2002). Many of these proteins do not accumulate in the cell in the absence of their cognate ligands. It has been suggested that this requirement of ligand binding for a disorder-to-order transition may increase binding rates, for a faster response to the stimulus, by increasing the likelihood that initial long-range interactions will occur (Shoemaker *et al.* 2000). The incorporation of ligand into the folding process may also optimize the specificity of the protein–ligand interaction (Dyson & Wright 2002). The term ‘intrinsically unstructured’ was coined by Dyson & Wright (2002) to signify the importance of these proteins in understanding cellular processes as well as protein folding mechanisms.

There is convincing evidence that TraR is a so-called intrinsically unstructured protein, as it requires 3-oxo-C8 HSL for stability and accumulation *in vivo*. When TraR is strongly overexpressed in either *Escherichia coli* or *A. tumefaciens* it does accumulate, but forms insoluble inclusion bodies. This led one group to suggest that apo-TraR is sequestered in the cellular membrane, and is released (and thus able to bind to DNA) only upon association with 3-oxo-C8 HSL (Qin *et al.* 2000). However, if the protein is only mildly expressed, then it does not accumulate in the absence of 3-oxo-C8 HSL but is in fact degraded by the cytoplasmic proteases (Zhu & Winans 2001). This led to the alternative suggestion that TraR requires 3-oxo-C8 HSL for stabilization against proteolysis. Therefore, 3-oxo-C8 HSL must trigger some conformational change in the protein upon binding. Furthermore, in the same study it was demonstrated with pulse-chase experiments that the apo-protein was targeted for proteolysis extremely rapidly. This led to the suggestion that 3-oxo-C8 HSL must stabilize TraR during translation, and therefore is likely to be part of the protein folding process itself. It was also demonstrated that purified apo-TraR is rapidly degraded by trypsin, whereas purified TraR–3-oxo-C8 HSL complexes were much more resistant to degradation (cleaving only at an inter-domain linker; Zhu & Winans 2001). This further supported the suggestion that the apo-protein is to at least some degree unfolded and must undergo a disorder-to-order transition upon 3-oxo-C8 HSL binding.

The data described above suggested that if inducing concentrations of 3-oxo-C8 HSL are present, TraR is stabilized in the cell and can activate transcription of target genes (Zhu & Winans 1999, 2001). However, the activity of stable TraR–3-oxo-C8 HSL complexes can still be directly inhibited by two different proteins, TraM and TrlR. On the octopine-type Ti plasmid, the

traM gene is adjacent to *traR*, just beyond the end of the *occ* operon (figure 1; Fuqua *et al.* 1995; Hwang *et al.* 1995). The fact that *traR* and *traM* are linked also suggests that *traM* and its function in the LuxR–LuxI system may be conserved. In fact, it has been shown that *traM* is even associated with these systems on the symbiosis megaplasmids of the rhizobia (He *et al.* 2003). For both the nopaline and the octopine-type Ti plasmids, it was shown that a null mutation in *traM* results in responses to lower concentrations of 3-oxo-C8 HSL than wild-type, and causes a hyperconjugal phenotype, while *traM* overexpression has the opposite effect (Fuqua *et al.* 1995; Hwang *et al.* 1995). Thus, it is clear that the *traM* gene product somehow attenuates TraR activity. Subsequent studies showed that the anti-activation effect of TraM is through direct protein–protein interactions with TraR–3-oxo-C8 HSL complexes (Hwang *et al.* 1999; Swiderska *et al.* 2001).

The adaptive significance of TraM is not entirely clear. In both nopaline- and octopine-type plasmids, it has been demonstrated that *traM* is activated by TraR in the presence of 3-oxo-C8 HSL, thus forming a negative feedback loop (Fuqua *et al.* 1995; Hwang *et al.* 1995). The results of a recent gene expression study using microarrays suggest that *traM* is also modestly induced in the presence of plant-released phenolics (remember that these compounds activate the *vir* genes of the Ti plasmid; Cho & Winans 2005). Perhaps this is a mechanism to avoid concurrent expression and activity of the *vir* and conjugation systems. Both have a type IV secretion system that is required for DNA transfer. It is possible that these two systems could interfere with each other if expressed at the same time in the cell.

The TrlR inhibitor of TraR activity is only known to be associated with octopine-type Ti plasmids. The *trlR* gene is near the distal end of the *mot* operon, which includes the genes required for mannopine uptake (figure 1; Oger *et al.* 1998; Zhu & Winans 1998). The expression of this operon is activated only in the presence of mannopine, possibly via the MocR protein (Oger *et al.* 1998; Zhu & Winans 1998). Mannopine has been shown to attenuate conjugation in octopine-type Ti plasmids, while conjugation can be restored by disrupting *trlR* (Oger *et al.* 1998; Zhu & Winans 1998). In addition, expression of *trlR* from a constitutive promoter also results in a decrease in conjugation (Chai *et al.* 2001). These experiments demonstrate that the negative effect of mannopine on conjugation is specifically through activation of *trlR* gene expression. Interestingly, it was also found that favoured catabolites, including succinate, glutamine and tryptone, block *trlR* expression (Chai *et al.* 2001). This led to the speculation that TrlR functions to attenuate the energetically expensive process of conjugation when nutrients are limiting.

4. BIOCHEMICAL AND STRUCTURAL STUDIES OF THE TraR–TraI SYSTEM

(a) *TraI: the autoinducer synthase*

In 1991, it was predicted that the LuxI-type autoinducer synthases would use *S*-adenosylmethionine (SAM) and either acyl-ACP or acyl-CoA as the precursors for the homoserine lactone and fatty acid

tail of the AHLs, respectively (Eberhard *et al.* 1991). This prediction was later confirmed using the purified histidine-tagged TraI protein (More *et al.* 1996). To define the exact substrates for 3-oxo-C8 HSL synthesis, the purified protein was mixed with a variety of potential substrates. It was determined that 3-oxooctanoyl-ACP was the sole fatty acid donor and that SAM was the sole precursor of the homoserine lactone group. This was the first demonstration of the activity of any LuxI-type protein *in vitro* in a purified system with defined substrates. Subsequent studies with other LuxI-type proteins, including LuxI and RhII, suggest that all of these proteins use similar substrates and have very similar reaction mechanisms (Val & Cronan 1998; Parsek *et al.* 1999).

High-resolution crystal structures are available for two LuxI-type proteins, EsaI of *Pantoea stewartii* and LasI of *Pseudomonas aeruginosa* (Watson *et al.* 2002; Gould *et al.* 2004). The reaction mechanisms of all of the proteins in this family are expected to be quite similar, and a number of residues in the active site of the proteins are conserved. A discussion of these proteins and AHL synthesis is beyond the scope of this review, and we refer the reader to another recent review (Pappas *et al.* 2004).

(b) *TraR: the autoinducer receptor*

The realization that TraR requires 3-oxo-C8 HSL for stability allowed overexpression and purification of stable TraR–3-oxo-C8 HSL complexes from *E. coli* for biochemical and structural studies. It was demonstrated *in vitro* with purified TraR–3-oxo-C8 HSL complexes that 3-oxo-C8 HSL binds to TraR monomers in a 1 : 1 mole ratio and that these complexes form homodimers in solution (Qin *et al.* 2000; Zhu & Winans 2001). It was also shown, with gel shift and footprint assays, that TraR–3-oxo-C8 HSL complexes bind, as dimers, to 18 bp sequences (called *tra* boxes) on the Ti plasmid with high affinity and specificity (Zhu & Winans 1999).

Two high-resolution crystal structures of TraR–3-oxo-C8 HSL complexes bound to *tra* box DNA were published in the same year, and to date they are the only structures of LuxR-type proteins available (Vannini *et al.* 2002; Zhang *et al.* 2002b). As one would expect, the structures are nearly identical, except for differences in the orientation of a few side chains at the surface of the protein. These structures of TraR confirmed earlier predictions that the protein binds to DNA as a dimer and that each monomer has two domains, an N-terminal 3-oxo-C8 HSL-binding domain and a C-terminal DNA-binding domain (figure 3a). The N-terminal domain has an α – β – α sandwich structure, with one molecule of 3-oxo-C8 HSL embedded between the β -sheet and a layer of α -helix (Vannini *et al.* 2002; Zhang *et al.* 2002b). In fact, the ligand is completely engulfed in the core of the protein and protected from solvent. Therefore, it is quite likely that the pheromone is incorporated during the protein folding process itself as previously predicted, or that major structural rearrangements must occur in TraR upon 3-oxo-C8 HSL binding. The contacts themselves between TraR and 3-oxo-C8 HSL are extensive, and include hydrophobic packing interactions between residues of TraR and the acyl chain. The polar groups

Figure 3. Ribbon model of a TraR dimer, complexed with 3-oxo-C8 HSL, bound to *tra* box DNA. One monomer of the dimer is in dark grey, the other in light grey and the DNA is black. One molecule of 3-oxo-C8 HSL, shown using space-filling atoms, is bound in the N-terminal domain (NTD) of each monomer, while the C-terminal domain (CTD) of each monomer is bound to DNA. (a) The helices of the NTD (helix 9) that form the dimerization interface are highlighted, and helix 13 of each CTD that aid in dimerization. The recognition helix of the HTH, helix 12, of each monomer is also labelled. Note that each binds in the major groove of each half site of the *tra* box. The N-terminus (N) of the left monomer and the C-termini (C) of both are labelled. The unstructured linker between the NTD and CTD of the right monomer was disordered in the crystal, but the linker of the left monomer is visible. (b) A side view of the model shown in (a), to illustrate the overall asymmetry of the dimer. Coordinates from Vannini *et al.* (2002) and Zhang *et al.* (2002b).

of the 3-oxo-C8 HSL make hydrogen bonds with residues in the binding pocket, such that the partial charges do not disrupt the integrity of the domain core (Vannini *et al.* 2002; Zhang *et al.* 2002b). These intimate and extensive contacts further support the model that 3-oxo-C8 HSL stabilizes the TraR protein *in vivo* by participating in the protein folding process itself.

The smaller C-terminal domain of each monomer is a four-helix bundle (figure 3a; Vannini *et al.* 2002; Zhang *et al.* 2002b). The second and third helices of this domain are a typical bacterial helix-turn-helix (HTH) DNA-binding motif (Nelson 1995). In fact, the amino acid sequence and the structure of this domain are highly conserved and place the LuxR family within the larger NarL–FixJ superfamily of bacterial transcription regulators (Fuqua & Greenberg 2002). All of these proteins have similar DNA-binding domains, but differ in their signal transduction domains. The high-resolution structures of only two additional proteins in this superfamily have been determined. One is the structure of the NarL DNA-binding domain, also crystallized with its DNA-binding site, and the other is the smaller protein GerE of *Bacillus subtilis*, which lacks a signal-binding domain altogether (Ducros *et al.* 2001; Maris *et al.* 2002). The DNA-binding domains of the otherwise distantly related TraR, NarL and GerE proteins superimpose extremely well with very little deviation.

There are two dimerization interfaces in the TraR–3-oxo-C8 HSL dimer (figure 3a). The most extensive of these is composed of a long α -helix in the N-terminal domain of each subunit that is parallel with the same helix of the opposite subunit (Vannini *et al.* 2002; Zhang *et al.* 2002b). A number of residues buried at this interface appear to contribute to dimer formation, a

finding confirmed by mutational analysis (Luo *et al.* 2003). A less extensive dimerization interface occurs between the C-terminal helices of the DNA-binding domain of each monomer. The N- and C-terminal domains of each monomer are connected by a 12-residue unstructured linker. Owing to this flexible linker and the fact that the dimerization interface is not continuous between the two monomers, there is extensive flexibility of the N-terminal domains relative to the C-terminal domains of the dimer. This flexibility resulted in a pronounced asymmetry in the crystal structure. The C-terminal domains of each dimer have a twofold axis of symmetry, and the N-terminal domains also have an axis of twofold symmetry, but these two axes actually lie at a 90° angle to each other (figure 3b). This flexibility has been proposed to play a role at divergently transcribed promoters (described in §5), although this remains to be determined.

In both of the structures the protein was crystallized with the 18 bp consensus *tra* box, which has a perfect dyad symmetry and is the strongest binding site for TraR (Vannini *et al.* 2002; Zhang *et al.* 2002b). The binding site is a regular B-form DNA, but with a smooth 30° bend towards the protein. Although this bend is modest when compared with that of other transcription factors, it still results in an increase in the buried surface area between the protein and the DNA (Jones *et al.* 1999). The protein–DNA interface is quite extensive, however, the only sequence-specific contacts are mediated by three amino acid residues of the recognition helix (the second helix of the HTH) and the edges of only four bases in the major groove of each *tra* box half site (figure 4; Vannini *et al.* 2002; Zhang *et al.* 2002b). However, many non-specific contacts between TraR and the DNA backbone also occur on either side of the major groove of each half site, and a number of these individual interactions are critical for binding affinity (White & Winans *in press*). At the centre of the *tra* box, the minor groove (separating the major groove of each half site) faces the protein and is not contacted by TraR. However, the bases of this ‘central spacer’ are still important for the protein–DNA interactions, most probably through their effects on DNA structure and flexibility (White & Winans *in press*).

There is significant interest in understanding the specificity of the interactions between LuxR-type receptors and their cognate AHLs, and how AHL binding converts these receptors from inactive to active forms. As described above, 3-oxo-C8 HSL is required for TraR stability against proteolysis (Zhu & Winans 2001). In addition it has been shown that when TraR is expressed at native levels *in vivo*, it binds to 3-oxo-C8 HSL with very high specificity (Zhu *et al.* 1998). In other words, TraR is able to distinguish its cognate ligand from a variety of other AHLs, even those that have very similar structures to 3-oxo-C8 HSL. The polar contacts in the binding pocket include four hydrogen bonds between the residues of TraR and the polar groups of 3-oxo-C8 HSL (figure 5). Two mutational studies of TraR confirmed that these hydrogen bonds are critical to 3-oxo-C8 HSL binding and stabilization of the protein (Luo *et al.* 2003; Chai & Winans 2004). One of these studies

Figure 4. Interactions between TraR and DNA. (a) A model of contact surfaces between TraR and the *tra* box. Specific contacts between residues of TraR (a valine and two arginines in each monomer) and four bases in the major groove are in dark grey. Interactions between TraR and the DNA backbone (non-specific) are white. (b) TraR interactions with the *tra* box. (White & Winans in press).

further considered the role of the 3-oxo group (which is not common to all AHLs) in specificity of binding (Chai & Winans 2004). This group makes a water-mediated hydrogen bond to a threonine residue in the binding pocket of TraR. Point mutations of the threonine residue were constructed in an attempt to increase hydrophobicity, which was predicted to exclude the water molecule from the binding pocket and therefore alter specificity for a 3-unsubstituted AHL (C8-acyl homoserine lactone). These mutations did result in an observable effect, although this was to broaden rather than alter the specificity of binding. In the same study, point mutations were also constructed in an attempt to change the preference of binding to AHLs with fatty acid tails shorter than eight carbons, by increasing the hydrophobic bulk in the binding pocket. A number of these mutations did result in altered specificity, however the stability of these mutants was also decreased. Although strongly altered specificity mutants were not identified in this study, the results predicted that the residues of the binding pocket play dual roles in ligand binding and stability or folding of the N-terminal domain. This really comes as no surprise, as these residues are buried in the

hydrophobic core of the protein and therefore must be important for protein folding and stabilization.

(c) *The anti-activators: TrlR and TraM*

Previously, we introduced TraM as an inhibitor of TraR activity (Fuqua *et al.* 1995; Hwang *et al.* 1995). A number of studies have been published on the activity of TraM, including two crystal structures of the protein (Chen *et al.* 2004; Vannini *et al.* 2004). Prior to the structural studies, TraM had been demonstrated using yeast two-hybrid assays and far western immunoblots to interact directly with TraR (Hwang *et al.* 1999). The same study used deletion and point mutations to show that the protein-protein interactions occurred at the C-terminal regions of both proteins. In an *in vitro* system with purified TraR and histidine-tagged TraM, it was shown that TraM can both block binding of TraR to *tra* box DNA and also disrupt preformed TraR-DNA complexes (Luo *et al.* 2000). These data led to the suggestion that TraM binds directly to the DNA-binding surface of TraR, making it inaccessible to DNA. In support of this argument, TraR-TraM complexes form with high affinity (in the nanomolar range) and are quite stable (Swiderska *et al.* 2001).

Figure 5. Interactions in the ligand-binding pocket of the TraR N-terminal domain. An α -helix and β -strand of TraR are shown, with critical residues and the 3-oxo-C8 HSL shown at the centre. Hydrogen bonds and their lengths (in angstroms) are shown. One water molecule is bound in the ligand-binding pocket, and coordinates interactions between a threonine residue and the 3-oxo group of 3-oxo-C8 HSL. See the text for details. Structural coordinates from Vannini *et al.* (2002) and Zhang *et al.* (2002b).

However, the ratio of TraR monomers to TraM monomers in TraR–TraM complexes was not so clear. Two different studies both used gel filtration chromatography to determine the mass of TraR–TraM complexes. The results from one study (Chen *et al.* 2004) suggested that one TraR monomer binds to one or two TraM monomers, while the results of the other study (Vannini *et al.* 2004) were consistent with two TraR dimers binding to two TraM dimers. These discrepancies must be due to differences in the experimental conditions. Another intriguing addition to the possible mechanism of TraR inhibition by TraM is that binding and inactivation may occur in sequential steps, as determined from studies using several TraM point mutants (Swiderska *et al.* 2001).

In both of the structural studies published, TraM was crystallized in the absence of TraR (Chen *et al.* 2004; Vannini *et al.* 2004). TraM crystallized as a dimer, with each subunit consisting largely of two antiparallel α -helices. A significant hydrophobic interface is buried at the interface between the two subunits. This extensive dimerization interface had also been confirmed in a study using deletion mutants of TraM (Qin *et al.* 2004b). The two groups that crystallized TraM published different models of how TraR–TraM interactions might disrupt TraR–DNA interactions. One model suggests that TraR and TraM homodimers may dissociate upon binding to each other so that the TraR–TraM anti-activation complex can form (Chen *et al.* 2004). This is consistent with mutational studies that showed that residues of TraM that are important for initial binding to TraR are different from those required for TraR inactivation (Swiderska *et al.* 2001). Furthermore, when the two subunits of TraM dimers are covalently cross-linked to

each other, the altered dimers are still able to bind TraR but are not able to disrupt DNA binding (Chen *et al.* 2004). A different model proposed that two dimers of TraM bind to two dimers of TraR, such that the TraM dimers are ‘clamped’ between the TraR dimers (Vannini *et al.* 2004). This complex would be expected to physically inhibit TraR dimers from binding to *tra* box DNA. Structural studies of TraR–TraM complexes may resolve the issue of how TraM inactivates TraR. However, static structures are not expected to provide a complete picture, as TraM binding to TraR and inactivation of TraR are expected to occur in sequential steps rather than simultaneously.

The other anti-activator of TraR activity, TrlR, has a much less complicated mechanism of TraR inhibition. The *trlR* gene is thought to have originated from a gene duplication event of *traR* itself (Oger *et al.* 1998). This gene is virtually identical to *traR* except for a single frame-shift difference upstream of the C-terminal DNA-binding domain. Although TrlR cannot activate *tra* genes, correction of the frame-shift mutation by site-directed mutagenesis results in an active protein (Zhu & Winans 1998). The similarity of TrlR to the TraR N-terminal domain led to the suggestion that TrlR inhibits TraR activity directly through formation of inactive heterodimers (which would have only one functional DNA-binding domain rather than two). Like TraR, TrlR requires 3-oxo-C8 HSL for solubility, and inactive TrlR–TraR heterodimers were shown to form *in vitro* using purified proteins (Chai *et al.* 2001). However, genes identical to *trlR* have been identified on a number of octopine-type Ti plasmids, indicating that the truncation is not a laboratory artefact (Zhu & Winans 1998).

(d) Regulation through signal depletion: 3-oxo-C8 HSL turnover

In addition to inhibition of TraR activity through TraM and TrlR, the products of the *attKLM* operon are also thought to inhibit the quorum-sensing system, although through an entirely different mechanism. The *attKLM* genes are located on the ‘cryptic’ plasmid pAtC58 and are the only known genes that are involved in the quorum-sensing system that are not on the Ti plasmid (Goodner *et al.* 2001; Wood *et al.* 2001). Interest in *attKLM* stemmed from a recent study in which the AttM-like protein AiiA of *Bacillus cereus* was shown to hydrolyze the lactone ring of AHLs (Dong *et al.* 2001). This lactonase activity results in the formation of *N*-acyl homoserines, which are inactive as signal molecules. AttM of *A. tumefaciens* was shown by two different groups to have the same lactonase activity on AHLs (Zhang *et al.* 2002a; Carlier *et al.* 2003).

The *attKLM* operon is repressed by the product of the divergently transcribed *attJ* gene. In an *attJ* null mutant, 3-oxo-C8 HSL did not accumulate to appreciable levels (Zhang *et al.* 2002a). However, it was not clear whether AttM was selected for this role. In one study, the *attKLM* operon in strain A6 was induced only at high population densities, and a later study by the same group suggested that carbon or nitrogen starvation and the ppGpp stress response are involved in *attKLM* regulation (Zhang *et al.* 2002a, 2004). However, in studies with *A. tumefaciens* strain C58,

a population-density effect on *attKLM* induction was not detected (Carlier *et al.* 2004; Y. Chai & S. C. Winans 2005, unpublished data). The difference between these studies could be due to a difference in the strains used.

As *attM* had been shown to be important for 3-oxo-C8 HSL depletion (Zhang *et al.* 2002a), a number of different groups tested AHLs for activation of the *attKLM* operon, expecting that these compounds might inactivate AttJ (Zhang *et al.* 2002a; Carlier *et al.* 2004; Chai *et al.* in press). However, none of the AHLs that were tested had any effect on *attKLM* induction. This finding suggested that perhaps AHLs are not the natural substrates of AttKLM.

In a recent study, a number of different γ -butyrolactones (GBLs) were tested as possible AttKLM substrates, as AttM was known to be a γ -butyrolactonase (Carlier *et al.* 2004). Only one of the compounds tested, GBL, could be used as the sole carbon source for growth of *A. tumefaciens* strain C58, and this growth was dependent on a functional *attKLM* operon. The predicted intermediates of GBL degradation via AttKLM were γ -hydroxybutyrate (GHB), from activity of AttM in opening the lactone ring, and succinate semialdehyde (SSA) and succinic acid (an intermediate of the tricarboxylic acid cycle) from activities of AttL and AttK, respectively (figure 6). A wild-type strain of C58 was able to grow on both GBL and GHB as the sole carbon source, while the *attKLM* null mutant could not (Carlier *et al.* 2004). In the same study, it was also reported that GBL and the intermediates GHB and SSA were all efficient inducers of an *attK::lacZ* fusion in *A. tumefaciens*. Growth of the wild-type C58 strain on a medium supplemented with the *attKLM* inducers (GBL, GHB or SSA) inhibited accumulation of 3-oxo-C8 HSL, while growth without these inducers did not. A direct and negative effect of GBL on the affinity of purified AttJ for *attKLM* promoter DNA has also been demonstrated recently (Chai *et al.* in press). Furthermore, *in vitro* experiments with purified proteins and defined substrates confirmed the predicted pathway of GBL degradation via AttKLM as described previously, and also demonstrated that the 3-oxo-C8 HSL degradation pathway is probably similar (figure 6; Chai *et al.* in press). These experiments strongly suggest that GBL is the substrate for which this catabolic operon was selected.

The adaptive significance of this pathway to *A. tumefaciens* is not clear. It is possible that *A. tumefaciens* may be using GBL or very similar compounds released by other soil bacteria as nutrients. If this were the case, then AttKLM could degrade AHLs produced by other nearby soil bacteria, but this would not affect the quorum-sensing system of *A. tumefaciens* itself, as *traR* is induced only in the presence of opines in or near infected plant tissue. On the other hand, it has been reported that GBL can be detected in at least some plants, and that the AttK and AttM proteins accumulate when *A. tumefaciens* is exposed to roots of tomato (Lee & Shibamoto 2000; Rosen *et al.* 2003). Further studies to assess the role of 'quorum-quenching' in the TraR–TraI system during plant colonization and growth near crown gall tumours will be quite interesting.

Figure 6. GBL degradation pathway via AttKLM (Carlier *et al.* 2004; Chai *et al.* in press). The pathway for 3-oxo-C8 HSL degradation is thought to be similar. At least the first step can result in hydrolysis of the lactone ring through activity of AttM.

5. TraR AS AN ACTIVATOR OF TRANSCRIPTION

As mentioned previously, all of the known TraR-regulated genes occur only on the Ti plasmids. These include the *tra* and *trb* genes, which are involved in conjugal transfer, the *rep* genes, which are required for vegetative replication and plasmid partitioning into daughter cells, and *traM*. The operon structure, promoters and *tra* boxes for activation of these genes have all been described on the octopine-type Ti plasmid (figure 7). The *tra* genes, required for DNA transfer and replication, are arranged in two operons: *traAFBH* and *traCDG-yci* (Fuqua & Winans 1996a). These operons are divergently transcribed from each other, and the origin of replication is predicted to be in the intergenic region between them. The *trb* genes of the *traI-trb* operon are involved in mating pair formation and the type IV secretion system for DNA transfer (Fuqua & Winans 1996a). Note that the 3-oxo-C8 HSL synthase, *traI*, is the first gene of this operon. The activation of *traI* expression by TraR-3-oxo-C8 HSL complexes represents a positive feedback loop, as has been described for a number of other LuxR–LuxI-type systems (Whitehead *et al.* 2001). The *traI-trb* operon is divergently transcribed from the *repABC* operon (Fuqua & Winans 1996a; Pappas & Winans 2003a).

There are a total of seven TraR-dependent promoters and four *tra* boxes that have been identified for the five operons described previously (figure 7; Fuqua & Winans 1996a; Pappas & Winans 2003a; C. Fuqua 2005, personal communication). Both *tra* operons are divergently transcribed from one *tra* box, called *tra* box I (which is also the consensus sequence). At both of these promoters the *tra* box is centred approximately 45 nucleotides upstream from the transcription start site and overlaps the –35 element of each promoter. This type of promoter structure, with

Figure 7. Transcription activation at TraR-dependent promoters on the octopine-type Ti plasmid. The *tra* boxes are marked as open squares and correspond to *tra* boxes I, II, III and IV. Thick bars represent both the -10 and the -35 elements of the promoter, and the transcription start sites are marked with arrows. The four promoters of the *rep* operon are labelled P_1 – P_4 , as described in the text. Activation at P_4 by phospho-VirG and repression of the same promoter by RepA–RepB complexes are also shown. Only the first gene (or part of the first gene) is shown for each operon.

the activator-binding site overlapping the distal end of the RNAP-binding site, is reminiscent of class II promoters, first described for CRP (Busby & Ebright 1999). The *traI*–*trb* and one *repABC* promoter (*repAP1*) are divergently transcribed from *tra* box II. Both of these promoters are also class II promoters. An additional TraR-dependent class II promoter (*repAP3*) has been identified for *repABC*, activated from *tra* box III (between *tra* box II and *rep*). There are also two class I TraR-dependent promoters on the octopine-type Ti plasmid. At this type of promoter, the activator binds further upstream from the transcription start site, but on the same face of the DNA as RNAP (Busby & Ebright 1999). At both the *traM* promoter, activated from *tra* box IV, and the *repAP2*, activated from *tra* box II, the *tra* box is centred at -66 nucleotides from the transcription start site. The *tra* boxes themselves are highly conserved. The affinities of TraR–3-oxo-C8 HSL complexes for *tra* boxes I, II and III are highly similar and in the nanomolar range (Zhu & Winans 1999; Pappas & Winans 2003a). The *tra* box IV is the least conserved of all four *tra* boxes, perhaps explaining the observation that the *traM* promoter is expressed more weakly than any other (White & Winans 2005; C. Fuqua 2005, personal communication).

The promoter architecture of *repABC* is rather complex, and includes an additional promoter that is not TraR-dependent, ensuring that plasmid maintenance and partitioning still occur when TraR is inactive (Pappas & Winans 2003b). This promoter (*repAP4*) is the closest of all four to *rep*, and is negatively regulated by RepA and RepB (Pappas & Winans 2003b; Chai & Winans 2005b). These two proteins are involved in plasmid partitioning, but a recent study has shown that they also form a nucleoprotein complex that autorepresses P_4 (Chai & Winans 2005b). RepA binds to DNA at P_4 , RepB binds to DNA between the *repA* and

repB genes, and contacts also form between RepA and RepB, possibly resulting in the formation of a repression loop (Pappas & Winans 2003b; Chai & Winans 2005b). In addition, a small antisense RNA (*repE*, encoded between *repB* and *repC*) attenuates the expression of *repC*, most likely through forming a complex with the *rep* transcript to terminate transcription directly or by blocking translation (Chai & Winans 2005a). RepC is required for plasmid replication initiation (Ramirez-Romero *et al.* 1997).

Surprisingly, it was recently noticed in expression studies using microarrays that the *repABC* operon is also induced in the presence of acetosyringone (AS; Cho & Winans 2005b). This compound is a plant-released phenolic that activates the *vir* genes via the VirA–VirG two-component system. AS-induced *rep* expression required activated VirG. The activation of *rep* via phospho-VirG enhances Ti plasmid copy number approximately fourfold (Cho & Winans 2005b), while activation via TraR–3-oxo-C8 HSL increases the copy number approximately eightfold (Pappas & Winans 2003a). A positive effect of TraR–3-oxo-C8 HSL complexes on *rep* transcription and Ti plasmid copy number in nopaline-type Ti plasmids has also been documented (Li & Farrand 2000). In fact, the genetic linkage of *rep* and *traI*–*trb* is strongly conserved in Ti plasmids, and even in some plasmids of *Rhizobium* sp. (Li & Farrand 2000; Zhu *et al.* 2000). Furthermore, all Ti plasmids studied have identifiable *tra* boxes in the intergenic region between *traI*–*trb* and *rep*. Therefore, it is expected that activation of Ti plasmid replication through the quorum-sensing system is a conserved feature.

The activation at TraR-dependent promoters on the octopine-type Ti plasmid requires only promoter DNA, TraR–3-oxo-C8 HSL complexes and $\sigma 70$ -RNAP

(Zhu & Winans 1999). Therefore, TraR is expected to make direct contacts with RNAP, and these contacts should serve to recruit polymerase to the promoter. At class II promoters, where an activator overlaps the -35 element, the activator is positioned to make multiple contacts with RNAP (Busby & Ebright 1999). However, at class I promoters, transcription activators are thought to recruit RNAP to the promoter solely through contacting the C-terminal domain of the α -subunit (α CTD). This domain, which also contacts DNA, is connected to the rest of core-RNAP by a flexible linker. As described previously, TraR can activate transcription at both class I and class II promoters, suggesting that TraR must contact the CTD of the α -subunit of RNAP. Two surface-exposed residues on the N-terminal domain of TraR have been identified that are required for activation but not DNA binding, and are thought to contact specifically the α CTD (Luo & Farrand 1999; Qin *et al.* 2004a). A larger potential RNAP contact site, or activating region, which includes a total of six residues, has been identified on the surface of the C-terminal domain of TraR (White & Winans 2005). Point mutations of residues in this patch disrupt activation but not DNA binding. It is interesting to note that this activating region overlaps with a patch on the surface of LuxR that is also critical for activation (Egland & Greenberg 2001; White & Winans 2005). However, in the case of LuxR, this region is predicted to contact $\sigma 70$, while this same region on TraR is predicted to contact the α CTD of RNAP, based on its importance for activation at both class I and class II promoters.

It is unlikely that TraR would have more than one activating region that contacts the CTD of the α -subunit. However, due to the high degree of flexibility of the N-terminal domains of the TraR dimer relative to the C-terminal domains, it is possible that the two critical residues of the N-terminal domain can approach the activating region identified on the C-terminal domain. Both sets of residues could together form a single contact site for the α CTD of RNAP. This potential and novel activating region, formed from two distinct sets of residues from different regions of TraR, could be important in the activity of TraR at divergent promoters, which are common on the Ti plasmid as described previously. However, further studies are required to completely understand TraR–RNAP interactions at TraR-dependent promoters.

6. PERSPECTIVES AND FUTURE STUDIES

In this review we have discussed the regulation of the quorum-sensing system in *A. tumefaciens*. Although many advances have been made in the biochemical and structural biology of TraI, TraR, the anti-activators TraM and TrlR, and lactonase turnover by AttKLM, many questions still remain. The mechanism of TraR inactivation by TraM is not clearly understood, but may be clarified with future structural studies of TraR–TraM complexes. Future biophysical studies that focus on the dynamics of TraR–3-oxo-C8 HSL interactions will also be fascinating and lend understanding to the role that 3-oxo-C8 HSL plays in TraR stability, and possibly even in protein folding. These studies could also reveal why some AHLs and AHL analogues inhibit

TraR activity (Zhu *et al.* 1998). Perhaps inhibitors are able to bind in the ligand pocket but do not induce the correct folding pathway to stabilize the protein against proteolysis. Structure determination of additional LuxR-type proteins will also allow for interesting comparative studies to understand the determinants of AHL specificity.

The recent studies described previously on AttKLM also prompt interesting questions about the substrates for which these enzymes were selected and about the significance of quorum-quenching by this pathway. 3-Oxo-C8 HSL degradation by AttKLM is ecologically important only if γ -butyrolactone and 3-oxo-C8 HSL are present at the same place and time. Only studies of the activity of both of these systems during plant colonization and pathogenesis will resolve these questions.

In this review, we have focussed on the molecular biology of the quorum-sensing system, but the adaptive significance of this system and the many regulators that are involved are not well understood. The quorum-sensing system of *A. tumefaciens* induces both vegetative replication and conjugal transfer of the Ti plasmid, but only in the presence of opines. This link between Ti plasmid replication, transfer and pathogenesis suggests that perhaps the role of TraR is to increase the copy number of the Ti plasmid in a population of plant-colonizing *A. tumefaciens*. Increased copy number could enhance both virulence and opine consumption, through a gene dosage effect. The recent evidence that the *rep* genes are also activated by phospho-VirG in response to AS further supports this argument.

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REFERENCES

- Beck von Bodman, S., Hayman, G. T. & Farrand, S. K. 1992 Opine catabolism and conjugal transfer of the nopaline Ti plasmid pTiC58 are coordinately regulated by a single repressor. *Proc. Natl Acad. Sci. USA* **89**, 643–647. (doi:10.1073/pnas.89.2.643)
- Binns, A. N. & Costantino, P. 1998 The *Agrobacterium* oncogenes. In *The Rhizobiaceae: molecular biology of model plant-associated bacteria* (eds H. P. Spaink, A. Kondorosi & P. J. Hooykaas), pp. 251–266. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Burr, T. J. & Otten, L. 1999 Crown gall of grape: biology and disease management. *Annu. Rev. Phytopathol.* **37**, 53–80. (doi:10.1146/annurev.phyto.37.1.53)
- Busby, S. & Ebright, R. H. 1999 Transcription activation by catabolite activator protein (CAP). *J. Mol. Biol.* **293**, 199–213. (doi:10.1006/jmbi.1999.3161)
- Carlier, A., Uroz, S., Smadja, B., Fray, R., Latour, X., Dessaux, Y. & Faure, D. 2003 The Ti plasmid of *Agrobacterium tumefaciens* harbors an *attM*-paralogous gene, *aiiB*, also encoding *N*-acyl homoserine lactonase activity. *Appl. Environ. Microbiol.* **69**, 4989–4993. (doi:10.1128/AEM.69.8.4989-4993.2003)
- Carlier, A., Chevrot, R., Dessaux, Y. & Faure, D. 2004 The assimilation of gamma-butyrolactone in *Agrobacterium tumefaciens* C58 interferes with the accumulation of the *N*-acyl-homoserine lactone signal. *Mol. Plant Microbe Interact.* **17**, 951–957.

- Chai, Y. & Winans, S. C. 2004 Site-directed mutagenesis of a LuxR-type quorum-sensing transcription factor: alteration of autoinducer specificity. *Mol. Microbiol.* **51**, 765–776. (doi:10.1046/j.1365-2958.2003.03857.x)
- Chai, Y. & Winans, S. C. 2005a A small antisense RNA downregulates expression of an essential replicase protein of an *Agrobacterium tumefaciens* Ti plasmid. *Mol. Microbiol.* **56**, 1574–1585.
- Chai, Y. & Winans, S. C. 2005b RepB protein of an *Agrobacterium tumefaciens* Ti plasmid binds to two adjacent sites between *repA* and *repB* for plasmid partitioning and autorepression. *Mol. Microbiol.* **58**, 1114–1129.
- Chai, Y., Tsai, C., Cho, H. & Winans, S. C. In Press. Reconstitution of the biochemical activities of the AttJ repressor and the attK, attL, and attM catabolic enzymes of *Agrobacterium tumefaciens*. *J. Bacteriol.*
- Chai, Y., Zhu, J. & Winans, S. C. 2001 TrlR, a defective TraR-like protein of *Agrobacterium tumefaciens*, blocks TraR function *in vitro* by forming inactive TrlR:TraR dimers. *Mol. Microbiol.* **40**, 414–421. (doi:10.1046/j.1365-2958.2001.02385.x)
- Chen, G., Malenkos, J. W., Cha, M. R., Fuqua, C. & Chen, L. 2004 Quorum-sensing antiactivator TraM forms a dimer that dissociates to inhibit TraR. *Mol. Microbiol.* **52**, 1641–1651. (doi:10.1111/j.1365-2958.2004.04110.x)
- Cho, H. & Winans, S. C. 2005 VirA and VirG activate the Ti plasmid *repABC* operon, elevating plasmid copy number in response to wound-released chemical signals. *Proc. Natl Acad. Sci. USA* **102**, 14843–14848. (doi:10.1073/Pnas.0503458102)
- Clare, B. G., Kerr, A. & Jones, D. A. 1990 Characteristics of the nopaline catabolic plasmid in *Agrobacterium* strains K84 and K1026 used for biological control of crown gall disease. *Plasmid* **23**, 126–137. (doi:10.1016/0147-619X(90)90031-7)
- Dessaux, Y., Petit, A. & Tempe, J. 1992 Opines in *Agrobacterium* biology. In *Molecular signals in plant-microbe communications* (ed. D. P. S. Verma), pp. 109–136. Ann Arbor, MI: CRC Press.
- Dessaux, Y., Petit, A., Farrand, S. K. & Murphy, P. J. 1998 Opines and opine-like molecules involved in plant/*Rhizobiaceae* interactions. In *The Rhizobiaceae* (eds H. P. Spaink, A. Kondorosi & P. J. Hooykaas), pp. 173–197. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Dong, Y. H., Wang, L. H., Xu, J. L., Zhang, H. B., Zhang, X. F. & Zhang, L. H. 2001 Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase. *Nature* **411**, 813–817. (doi:10.1038/35081101)
- Ducros, V. M., Lewis, R. J., Verma, C. S., Dodson, E. J., Leonard, G., Turkenburg, J. P., Murshudov, G. N., Wilkinson, A. J. & Brannigan, J. A. 2001 Crystal structure of GerE, the ultimate transcriptional regulator of spore formation in *Bacillus subtilis*. *J. Mol. Biol.* **306**, 759–771. (doi:10.1006/jmbi.2001.4443)
- Dyson, H. J. & Wright, P. E. 2002 Coupling of folding and binding for unstructured proteins. *Curr. Opin. Struct. Biol.* **12**, 54–60. (doi:10.1016/S0959-440X(02)00289-0)
- Eberhard, A., Longin, T., Widrig, C. A. & Stranick, S. J. 1991 Synthesis of the *lux* gene autoinducer in *Vibrio fischeri* is positively autoregulated. *Arch. Microbiol.* **155**, 294–297. (doi:10.1007/BF00252215)
- Egland, K. A. & Greenberg, E. P. 2001 Quorum sensing in *Vibrio fischeri*: analysis of the LuxR DNA binding region by alanine-scanning mutagenesis. *J. Bacteriol.* **183**, 382–386. (doi:10.1128/JB.183.1.382-386.2001)
- Ellis, J. G., Kerr, A., Petit, A. & Tempe, J. 1982 Conjugal transfer of nopaline and agropine Ti-plasmids—the role of agrocinosines. *Mol. Gen. Genet.* **186**, 269–273. (doi:10.1007/BF00331861)
- Engbrecht, J. & Silverman, M. 1984 Identification of genes and gene products necessary for bacterial bioluminescence. *Proc. Natl Acad. Sci. USA* **81**, 4154–4158. (doi:10.1073/pnas.81.13.4154)
- Engbrecht, J. & Silverman, M. 1987 Nucleotide sequence of the regulatory locus controlling expression of bacterial genes for bioluminescence. *Nucleic Acids Res.* **15**, 10 455–10 467. (doi:10.1093/nar/15.24.10455)
- Engbrecht, J., Nealson, K. & Silverman, M. 1983 Bacterial bioluminescence: isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* **32**, 773–781. (doi:10.1016/0092-8674(83)90063-6)
- Escobar, M. A. & Dandekar, A. M. 2003 *Agrobacterium tumefaciens* as an agent of disease. *Trends Plant Sci.* **8**, 380–386. (doi:10.1016/S1360-1385(03)00162-6)
- Fuqua, C. & Greenberg, E. P. 2002 Listening in on bacteria: acyl-homoserine lactone signalling. *Nat. Rev. Mol. Cell. Biol.* **3**, 685–695. (doi:10.1038/nrm907)
- Fuqua, W. C. & Winans, S. C. 1994 A LuxR–LuxI type regulatory system activates *Agrobacterium* Ti plasmid conjugal transfer in the presence of a plant tumor metabolite. *J. Bacteriol.* **176**, 2796–2806.
- Fuqua, C. & Winans, S. C. 1996a Conserved *cis*-acting promoter elements are required for density-dependent transcription of *Agrobacterium tumefaciens* conjugal transfer genes. *J. Bacteriol.* **178**, 435–440.
- Fuqua, C. & Winans, S. C. 1996b Localization of OccR-activated and TraR-activated promoters that express two ABC-type permeases and the *traR* gene of Ti plasmid pTiR10. *Mol. Microbiol.* **20**, 1199–1210. (doi:10.1111/j.1365-2958.1996.tb02640.x)
- Fuqua, C., Burbea, M. & Winans, S. C. 1995 Activity of the *Agrobacterium* Ti plasmid conjugal transfer regulator TraR is inhibited by the product of the *traM* gene. *J. Bacteriol.* **177**, 1367–1373.
- Gelvin, S. B. 2003 *Agrobacterium*-mediated plant transformation: the biology behind the “gene-jockeying” tool. *Microbiol. Mol. Biol. Rev.* **67**, 16–37. (doi:10.1128/MMBR.67.1.16-37.2003)
- Genetello, C., Van Larebeke, N., Holsters, M., De Picker, A., Van Montagu, M. & Schell, J. 1977 Ti plasmids of *Agrobacterium* as conjugative plasmids. *Nature* **265**, 561–563. (doi:10.1038/265561a0)
- Gonzalez, J. E. & Marketon, M. M. 2003 Quorum sensing in nitrogen-fixing rhizobia. *Microbiol. Mol. Biol. Rev.* **67**, 574–592. (doi:10.1128/MMBR.67.4.574-592.2003)
- Goodner, B. *et al.* 2001 Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. *Science* **294**, 2323–2328. (doi:10.1126/science.1066803)
- Gould, T. A., Schweizer, H. P. & Churchill, M. E. 2004 Structure of the *Pseudomonas aeruginosa* acyl-homoserine lactone synthase LasI. *Mol. Microbiol.* **53**, 1135–1146. (doi:10.1111/j.1365-2958.2004.04211.x)
- Hayman, G. T. & Farrand, S. K. 1988 Characterization and mapping of the agrocinosine–agrocinosin 84 locus on the nopaline Ti plasmid pTiC58. *J. Bacteriol.* **170**, 1759–1767.
- Hayman, G. T. & Farrand, S. K. 1990 *Agrobacterium* plasmids encode structurally and functionally different loci for catabolism of agrocinosine-type opines. *Mol. Gen. Genet.* **223**, 465–473. (doi:10.1007/BF00264455)
- He, X., Chang, W., Pierce, D. L., Seib, L. O., Wagner, J. & Fuqua, C. 2003 Quorum sensing in *Rhizobium* sp. strain NGR234 regulates conjugal transfer (*tra*) gene expression and influences growth rate. *J. Bacteriol.* **185**, 809–822. (doi:10.1128/JB.185.3.809-822.2003)
- Hwang, I., Li, P. L., Zhang, L., Piper, K. R., Cook, D. M., Tate, M. E. & Farrand, S. K. 1994 TraI, a LuxI homologue, is responsible for production of conjugation

- factor, the Ti plasmid *N*-acylhomoserine lactone auto-inducer. *Proc. Natl Acad. Sci. USA* **91**, 4639–4643. (doi:10.1073/pnas.91.11.4639)
- Hwang, I., Cook, D. M. & Farrand, S. K. 1995 A new regulatory element modulates homoserine lactone-mediated autoinduction of Ti plasmid conjugal transfer. *J. Bacteriol.* **177**, 449–458.
- Hwang, I., Smyth, A. J., Luo, Z. Q. & Farrand, S. K. 1999 Modulating quorum sensing by antiactivation: TraM interacts with TraR to inhibit activation of Ti plasmid conjugal transfer genes. *Mol. Microbiol.* **34**, 282–294. (doi:10.1046/j.1365-2958.1999.01595.x)
- Johnson, T. M. & Das, A. 1998 Organization and regulation of expression of the *Agrobacterium* virulence genes. In *The Rhizobiaceae: molecular biology of model plant-associated bacteria* (eds H. P. Spaink, A. Kondorosi & P. J. Hooykaas), pp. 265–279. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Jones, S., van Heyningen, P., Berman, H. M. & Thornton, J. M. 1999 Protein–DNA interactions: a structural analysis. *J. Mol. Biol.* **287**, 877–896. (doi:10.1006/jmbi.1999.2659)
- Kado, C. I. 1994 Promiscuous DNA transfer system of *Agrobacterium tumefaciens*: role of the *virB* operon in sex pilus assembly and synthesis. *Mol. Microbiol.* **12**, 17–22. (doi:10.1111/j.1365-2958.1994.tb00990.x)
- Kerr, A. 1969 Transfer of virulence between isolates of *Agrobacterium*. *Nature* **223**, 1175–1176. (doi:10.1038/2231175a0)
- Kerr, A. 1971 Acquisition of virulence by non-pathogenic isolates of *Agrobacterium radiobacter*. *Physiol. Plant Pathol.* **1**, 241–246.
- Kerr, A., Manigault, P. & Tempe, J. 1977 Transfer of virulence *in vivo* and *in vitro* in *Agrobacterium*. *Nature* **265**, 560–561. (doi:10.1038/265560a0)
- Lee, K. G. & Shibamoto, T. 2000 Antioxidant properties of aroma compounds isolated from soybeans and mung beans. *J. Agric. Food Chem.* **48**, 4290–4293. (doi:10.1021/jf000442u)
- Lessl, M. & Lanka, E. 1994 Common mechanisms in bacterial conjugation and Ti-mediated T-DNA transfer to plant cells. *Cell* **77**, 321–324. (doi:10.1016/0092-8674(94)90146-5)
- Lessl, M., Balzer, D., Pansegrau, W. & Lanka, E. 1992 Sequence similarities between the RP4 Tra2 and the Ti VirB region strongly support the conjugation model for T-DNA transfer. *J. Biol. Chem.* **267**, 20 471–20 480.
- Lewis, M. 2005 The lac repressor. *C. R. Biol.* **328**, 521–548. (doi:10.1016/j.crv.2005.04.004)
- Li, P. L. & Farrand, S. K. 2000 The replicator of the nopaline-type Ti plasmid pTiC58 is a member of the *repABC* family and is influenced by the TraR-dependent quorum-sensing regulatory system. *J. Bacteriol.* **182**, 179–188.
- Luo, Z. Q. & Farrand, S. K. 1999 Signal-dependent DNA binding and functional domains of the quorum-sensing activator TraR as identified by repressor activity. *Proc. Natl Acad. Sci. USA* **96**, 9009–9014. (doi:10.1073/pnas.96.16.9009)
- Luo, Z. Q., Qin, Y. & Farrand, S. K. 2000 The antiactivator TraM interferes with the autoinducer-dependent binding of TraR to DNA by interacting with the C-terminal region of the quorum-sensing activator. *J. Biol. Chem.* **275**, 7713–7722. (doi:10.1074/jbc.275.11.7713)
- Luo, Z. Q., Smyth, A. J., Gao, P., Qin, Y. & Farrand, S. K. 2003 Mutational analysis of TraR. Correlating function with molecular structure of a quorum-sensing transcriptional activator. *J. Biol. Chem.* **278**, 13 173–13 182. (doi:10.1074/jbc.M210035200)
- Maris, A. E., Sawaya, M. R., Kaczor-Grzeskowiak, M., Jarvis, M. R., Bearson, S. M., Kopka, M. L., Schroder, I., Gunsalus, R. P. & Dickerson, R. E. 2002 Dimerization allows DNA target site recognition by the NarL response regulator. *Nat. Struct. Biol.* **9**, 771–778. (doi:10.1038/nsb845)
- McFall, S. M., Chugani, S. A. & Chakrabarty, A. M. 1998 Transcriptional activation of the catechol and chlorocatechol operons: variations on a theme. *Gene* **223**, 257–267. (doi:10.1016/S0378-1119(98)00366-7)
- More, M. I., Finger, L. D., Stryker, J. L., Fuqua, C., Eberhard, A. & Winans, S. C. 1996 Enzymatic synthesis of a quorum-sensing autoinducer through use of defined substrates. *Science* **272**, 1655–1658. (doi:10.1126/science.272.5268.1655)
- Morris, R. O. 1990 Genes specifying auxin and cytokinin biosynthesis in prokaryotes. In *Plant hormones and their role in plant growth and development* (ed. P. J. Davies), pp. 636–655. Dordrecht, The Netherlands: Kluwer Academic Publishers.
- Nelson, H. C. 1995 Structure and function of DNA-binding proteins. *Curr. Opin. Genet. Dev.* **5**, 180–189. (doi:10.1016/0959-437X(95)80006-9)
- Oger, P. & Farrand, S. K. 2001 Co-evolution of the agrocinopine opines and the agrocinopine-mediated control of TraR, the quorum-sensing activator of the Ti plasmid conjugation system. *Mol. Microbiol.* **41**, 1173–1185. (doi:10.1046/j.1365-2958.2001.02584.x)
- Oger, P. & Farrand, S. K. 2002 Two opines control conjugal transfer of an *Agrobacterium* plasmid by regulating expression of separate copies of the quorum-sensing activator gene *traR*. *J. Bacteriol.* **184**, 1121–1131. (doi:10.1128/jb.184.4.1121-1131.2002)
- Oger, P., Kim, K. S., Sackett, R. L., Piper, K. R. & Farrand, S. K. 1998 Octopine-type Ti plasmids code for a mannopine-inducible dominant-negative allele of *traR*, the quorum-sensing activator that regulates Ti plasmid conjugal transfer. *Mol. Microbiol.* **27**, 277–288. (doi:10.1046/j.1365-2958.1998.00671.x)
- Pappas, K. M. & Winans, S. C. 2003a A LuxR-type regulator from *Agrobacterium tumefaciens* elevates Ti plasmid copy number by activating transcription of plasmid replication genes. *Mol. Microbiol.* **48**, 1059–1073. (doi:10.1046/j.1365-2958.2003.03488.x)
- Pappas, K. M. & Winans, S. C. 2003b The RepA and RepB autorepressors and TraR play opposing roles in the regulation of a Ti plasmid *repABC* operon. *Mol. Microbiol.* **49**, 441–455. (doi:10.1046/j.1365-2958.2003.03560.x)
- Pappas, K. M., Weingart, C. L. & Winans, S. C. 2004 Chemical communication in proteobacteria: biochemical and structural studies of signal synthases and receptors required for intercellular signalling. *Mol. Microbiol.* **53**, 755–769. (doi:10.1111/j.1365-2958.2004.04212.x)
- Parsek, M. R., Val, D. L., Hanzelka, B. L., Cronan Jr, J. E. & Greenberg, E. P. 1999 Acyl homoserine-lactone quorum-sensing signal generation. *Proc. Natl Acad. Sci. USA* **96**, 4360–4365. (doi:10.1073/pnas.96.8.4360)
- Piper, K. R., Beck von Bodman, S. & Farrand, S. K. 1993 Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. *Nature* **362**, 448–450. (doi:10.1038/362448a0)
- Piper, K. R., Beck Von Bodman, S., Hwang, I. & Farrand, S. K. 1999 Hierarchical gene regulatory systems arising from fortuitous gene associations: controlling quorum sensing by the opine regulon in *Agrobacterium*. *Mol. Microbiol.* **32**, 1077–1089. (doi:10.1046/j.1365-2958.1999.01422.x)
- Pohlman, R. F., Genetti, H. D. & Winans, S. C. 1994 Common ancestry between IncN conjugal transfer genes

- and macromolecular export systems of plant and animal pathogens. *Mol. Microbiol.* **14**, 655–668. (doi:10.1111/j.1365-2958.1994.tb01304.x)
- Qin, Y., Luo, Z. Q., Smyth, A. J., Gao, P., Beck von Bodman, S. & Farrand, S. K. 2000 Quorum-sensing signal binding results in dimerization of TraR and its release from membranes into the cytoplasm. *EMBO J.* **19**, 5212–5221. (doi:10.1093/emboj/19.19.5212)
- Qin, Y. P., Luo, Z. Q. & Farrand, S. K. 2004a Domains formed within the N-terminal region of the quorum-sensing activator TraR are required for transcriptional activation and direct interaction with RpoA from *Agrobacterium*. *J. Biol. Chem.* **279**, 40 844–40 851. (doi:10.1074/jbc.M405299200)
- Qin, Y., Smyth, A. J., Su, S. & Farrand, S. K. 2004b Dimerization properties of TraM, the antiactivator that modulates TraR-mediated quorum-dependent expression of the Ti plasmid *tra* genes. *Mol. Microbiol.* **53**, 1471–1485. (doi:10.1111/j.1365-2958.2004.04216.x)
- Ramirez-Romero, M. A., Bustos, P., Girard, L., Rodriguez, O., Cevallos, M. A. & Davila, G. 1997 Sequence, localization and characteristics of the replicator region of the symbiotic plasmid of *Rhizobium etli*. *Microbiology* **143**, 2825–2831.
- Rosen, R., Matthyse, A. G., Becher, D., Biran, D., Yura, T., Hecker, M. & Ron, E. Z. 2003 Proteome analysis of plant-induced proteins of *Agrobacterium tumefaciens*. *FEMS Microbiol. Ecol.* **44**, 355–360. (doi:10.1016/S0168-6496(03)00077-1)
- Schell, M. A. 1993 Molecular biology of the LysR family of transcriptional regulators. *Annu. Rev. Microbiol.* **47**, 597–626. (doi:10.1146/annurev.mi.47.100193.003121)
- Shoemaker, B. A., Portman, J. J. & Wolynes, P. G. 2000 Speeding molecular recognition by using the folding funnel: the fly-casting mechanism. *Proc. Natl Acad. Sci. USA* **97**, 8868–8873. (doi:10.1073/pnas.160259697)
- Stachel, S. E. & Zambryski, P. C. 1986 *virA* and *virG* control the plant-induced activation of the T-DNA transfer process of *A. tumefaciens*. *Cell* **46**, 325–333. (doi:10.1016/0092-8674(86)90653-7)
- Swiderska, A., Berndtson, A. K., Cha, M. R., Li, L., Beaudoin 3rd, G. M., Zhu, J. & Fuqua, C. 2001 Inhibition of the *Agrobacterium tumefaciens* TraR quorum-sensing regulator. Interactions with the TraM anti-activator. *J. Biol. Chem.* **276**, 49 449–49 458. (doi:10.1074/jbc.M107881200)
- Val, D. L. & Cronan Jr, J. E. 1998 *In vivo* evidence that S-adenosylmethionine and fatty acid synthesis intermediates are the substrates for the LuxI family of autoinducer synthases. *J. Bacteriol.* **180**, 2644–2651.
- Van Larebeke, N., Engler, G., Holsters, M., Van den Elsacker, S., Zaenen, I., Schilperoort, R. A. & Schell, J. 1974 Large plasmid in *Agrobacterium tumefaciens* essential for crown gall-inducing ability. *Nature* **252**, 169–170. (doi:10.1038/252169a0)
- Vannini, A., Volpari, C., Gargioli, C., Muraglia, E., Cortese, R., De Francesco, R., Neddermann, P. & Marco, S. D. 2002 The crystal structure of the quorum sensing protein TraR bound to its autoinducer and target DNA. *EMBO J.* **21**, 4393–4401. (doi:10.1093/emboj/cdf459)
- Vannini, A., Volpari, C. & Di Marco, S. 2004 Crystal structure of the quorum-sensing protein TraM and its interaction with the transcriptional regulator TraR. *J. Biol. Chem.* **279**, 24 291–24 296. (doi:10.1074/jbc.M401855200)
- Wang, L., Helmann, J. D. & Winans, S. C. 1992 The *A. tumefaciens* transcriptional activator OccR causes a bend at a target promoter, which is partially relaxed by a plant tumor metabolite. *Cell* **69**, 659–667. (doi:10.1016/0092-8674(92)90229-6)
- Watson, W. T., Minogue, T. D., Val, D. L., von Bodman, S. B. & Churchill, M. E. 2002 Structural basis and specificity of acyl-homoserine lactone signal production in bacterial quorum sensing. *Mol. Cell* **9**, 685–694. (doi:10.1016/S1097-2765(02)00480-X)
- White, C. E. & Winans, S. C. In Press. The quorum-sensing transcription factor TraR decodes its DNA binding site by direct contacts with DNA bases and by detection of DNA flexibility. *Mol. Microbiol.*
- White, C. E. & Winans, S. C. 2005 Identification of amino acid residues of the *Agrobacterium tumefaciens* quorum-sensing regulator TraR that are critical for positive control of transcription. *Mol. Microbiol.* **55**, 1473–1486. (doi:10.1111/j.1365-2958.2004.04482.x)
- Whitehead, N. A., Barnard, A. M., Slater, H., Simpson, N. J. & Salmond, G. P. 2001 Quorum-sensing in Gram-negative bacteria. *FEMS Microbiol. Rev.* **25**, 365–404. (doi:10.1111/j.1574-6976.2001.tb00583.x)
- Winans, S. C., Kerstetter, R. A. & Nester, E. W. 1988 Transcriptional regulation of the *virA* and *virG* genes of *Agrobacterium tumefaciens*. *J. Bacteriol.* **170**, 4047–4054.
- Wisniewski-Dye, F. & Downie, J. A. 2002 Quorum-sensing in *Rhizobium*. *Antonie Van Leeuwenhoek* **81**, 397–407.
- Wood, D. W. *et al.* 2001 The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. *Science* **294**, 2317–2323. (doi:10.1126/science.1066804)
- Young, J. M., Kuykendall, L. D., Martinez-Romero, E., Kerr, A. & Sawada, H. 2003 Classification and nomenclature of *Agrobacterium* and *Rhizobium*. *Int. J. Syst. Evol. Microbiol.* **53**, 1689–1695. (doi:10.1099/ijs.0.02762-0)
- Zhang, L., Murphy, P. J., Kerr, A. & Tate, M. E. 1993 *Agrobacterium* conjugation and gene regulation by N-acyl-L-homoserine lactones. *Nature* **362**, 446–448. (doi:10.1038/362446a0)
- Zhang, H. B., Wang, L. H. & Zhang, L. H. 2002a Genetic control of quorum-sensing signal turnover in *Agrobacterium tumefaciens*. *Proc. Natl Acad. Sci. USA* **99**, 4638–4643. (doi:10.1073/pnas.022056699)
- Zhang, R. G. *et al.* 2002b Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA. *Nature* **417**, 971–974. (doi:10.1038/nature00833)
- Zhang, H. B., Wang, C. & Zhang, L. H. 2004 The quorumone degradation system of *Agrobacterium tumefaciens* is regulated by starvation signal and stress alarmone (p)ppGpp. *Mol. Microbiol.* **52**, 1389–1401. (doi:10.1111/j.1365-2958.2004.04061.x)
- Zhu, J. & Winans, S. C. 1998 Activity of the quorum-sensing regulator TraR of *Agrobacterium tumefaciens* is inhibited by a truncated, dominant defective TraR-like protein. *Mol. Microbiol.* **27**, 289–297. (doi:10.1046/j.1365-2958.1998.00672.x)
- Zhu, J. & Winans, S. C. 1999 Autoinducer binding by the quorum-sensing regulator TraR increases affinity for target promoters *in vitro* and decreases TraR turnover rates in whole cells. *Proc. Natl Acad. Sci. USA* **96**, 4832–4837. (doi:10.1073/pnas.96.9.4832)
- Zhu, J. & Winans, S. C. 2001 The quorum-sensing transcriptional regulator TraR requires its cognate signaling ligand for protein folding, protease resistance, and dimerization. *Proc. Natl Acad. Sci. USA* **98**, 1507–1512. (doi:10.1073/pnas.98.4.1507)
- Zhu, J., Beaver, J. W., More, M. I., Fuqua, C., Eberhard, A. & Winans, S. C. 1998 Analogs of the autoinducer 3-oxooctanoyl-homoserine lactone strongly inhibit activity of the TraR protein of *Agrobacterium tumefaciens*. *J. Bacteriol.* **180**, 5398–5405.
- Zhu, J., Oger, P. M., Schrammeijer, B., Hooykaas, P. J., Farrand, S. K. & Winans, S. C. 2000 The bases of crown gall tumorigenesis. *J. Bacteriol.* **182**, 3885–3895. (doi:10.1128/JB.182.14.3885-3895.2000)