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Quorum Sensing and Plant Factors Combine to Induce Plasmid Transfer Among Rhizobacteria

Stephen K. Farrand*, Yinping Qin, and Zhao-Qing Luo. Departments of Crop Sciences and Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois USA 61801

Certain members of the genus *Agrobacterium* can induce neoplastic growths, called crown galls or hairy roots, on dicotyledonous plants. In addition to their cancer-like properties, crown gall tumors and hairy roots produce novel carbon compounds generically called opines. These compounds, which are released into the rhizosphere, can be utilized by the agrobacteria as sole sources of carbon and energy. The capacity to induce these tumors is conferred on the bacteria by large extrachromosomal elements, which in the case of *A. tumefaciens* which causes crown galls are called Ti plasmids. These plasmids also code for the genes responsible for the transport and catabolism by the bacteria of the opines produced by the tumors. Tumorigenesis is associated with the transfer of a defined portion of the Ti plasmid, called the T-region, from the bacterium to the plant by a process resembling bacterial conjugation. The genes required for such transfer are contained within the *vir* regulon of the Ti plasmid. Ti plasmids studied to date contain a second, completely independent transfer system, called *tra*, which mediates the conjugal transfer of the entire Ti plasmid from one bacterium to another. Phylogenetic analysis indicates that both transfer systems are chimeric, and that each arose independently of the other.

Both transfer systems are strongly regulated at the level of transcription by plant-produced signals. Expression of *vir* is triggered by phenolic compounds and monosaccharides produced by wounded plant cells. These compounds regulate the *vir* genes via a two component signal transduction system coded for by *virA* and *virG*. Induction of the *tra* genes, on the other hand, responds to a set of different signals, certain of the opines produced by the tumors that are induced by the bacterium (1). These so-called conjugal opines also regulate expression of the genes associated with their uptake and catabolism (1).

While the opines control expression of their catabolic gene systems directly, they are not the direct regulators of the genes involved in conjugal transfer. Rather, these genes, organized as three operons, *traAFB*, *traCDG* and *trb* (2,3) require the transcriptional activator TraR (4). TraR, in turn, requires as co-activator a small diffusible signal molecule, called *Agrobacterium* autoinducer (AAI), that is produced by the bacteria themselves (5,6). TraR is a member of the LuxR family of transcriptional activators, and AAI is an acyl-homoserine lactone [*N*-(3-oxo-octanoyl)-L-homoserine lactone] related to the autoinducer of Lux, called VAI [*N*-(3-oxo-hexanoyl)-L-homoserine lactone].

In this regard, conjugal transfer of the Ti plasmids is regulated by autoinduction (7). This gene regulatory system, first described for the control of bioluminescence in the marine symbiont *Vibrio fischeri* functions to couple the expression of gene systems to the population size of the donor in a process now called quorum sensing (8). The cells produce the autoinducer at a low level, and the signal passes out of the cells by diffusion or by an efflux system. Given the acyl side chain, the extracellular signal can also pass back into the cells via diffusion through the lipid bilayer. Because of continued synthesis, as the cells grow, the concentration of the autoinducer increases at a rate faster than the increase in population size. When the autoinducer reaches some

critical concentration, it activates the transcriptional activator, and the target genes are expressed. In sense, then, the bacteria measure their population size by gauging the amount of the autoinducer that has accumulated in the environment.

Studies indicate that Ti plasmid transfer is regulated by quorum sensing via the TraR/AAI autoinduction system (7). However, it certainly is the case that conjugal transfer also requires induction by the conjugal opine. Control by opines and by autoinduction is coupled by the simple expediency of placing expression of *traR* under the umbrella of the opine regulon. For the nopaline/agrocinopine-type Ti plasmids such as pTiC58 conjugation is induced by the sugar phosphodiester opines agrocinopines A and B (9). The *traR* gene of this Ti plasmid is a member of a five-gene operon that is negatively regulated by AccR, the master repressor that responds to the presence of the agrocinopine opines (10,11). AccR also negatively regulates the *acc* operon which is responsible for the transport and catabolism of this opine family (10). In the octopine/mannityl opine-type Ti plasmids such as pTiR10 and pTi15955 conjugation is induced by octopine. In this case, *traR* is part of a 14 gene operon that is positively regulated by OccR, a transcriptional activator that responds to octopine and related opines (12). Remarkably, while the *tra* system, and the *traR* genes of the two Ti plasmids are essentially identical, and while the strategy of regulation of *traR* expression by opines is the same, the genes responsible for opine control of *traR* expression and their organization are completely unrelated between the two Ti plasmids (11). Analysis of additional Ti plasmids indicates that opine regulation of transfer is a common theme, and that such control results from placing the *traR* gene of these elements within the opine regulon. In this regard, at least four systems in which *traR* is controlled by different opines have evolved independently (11, 13), and at least one of these systems is showing signs of divergence within its family. In all cases, however, the strategy results in a hierarchical regulatory cascade in which the Ti plasmid must perceive two signals before initiating conjugal transfer. The first signal, the conjugal opine must be present; this, we believe informs the Ti plasmid that the environment is conducive to horizontal dissemination of this genetic element. The second signal, the quorum-sensing autoinducer, advises the Ti plasmid that the time is right for transfer; that is, the donor size is optimum for the population dynamics associated with plasmid transfer in the environment of the crown gall tumor.

Although we have some understanding of the role of the acyl homoserine lactone in the biology of autoinduction, little is known about how this signal molecule converts TraR into its active form. Using a genetic test in which TraR functions as a repressor, interaction with AAI is required for TraR to bind the *tra* box, its cognate DNA recognition site (14). Similarly, using another genetic test, interaction with AAI is required for TraR to dimerize. Furthermore, certain mutants of TraR, most notably those deleted for regions of the C-terminus exert strong dominant negativity when co-expressed with the wild-type activator. These results suggest that active TraR exists in dimer form and that interaction with AAI is required for dimer formation.

Biochemical analysis of purified TraR supports this hypothesis. The active protein purified from *A. tumefaciens* elutes from a calibrated size exclusion column at an elution volume corresponding to the dimer form. This purified protein is tightly associated with AAI; prolonged dialysis is required to remove the homoserine lactone from the activator (15). Furthermore, removing AAI by dialysis is associated with conversion of TraR to the monomer form of the protein. Using a combination of genetic and biochemical analyses, we have located the regions

of TraR involved in signal binding and dimerization to the N-terminal portion of the protein. Within this region we have identified amino acid residues important for each process.

Finally, like LuxR (16), TraR appears to be associated with the inner membrane of the bacterium. Moreover, as assessed by cross-linking studies, in the absence of AAI virtually all of the detectable TraR is present in the membrane in monomer form. Interaction with AAI results in the appearance in both the membrane and the soluble fractions of the dimer form of the activator.

From these results we propose a model in which, when the quorum-sensing signal is low, TraR is preferentially localized to the cytoplasmic membrane in monomer form. As the amount of signal increases as function of population growth, the autoinducer concentration builds up in the membrane where it can interact with the monomer form of TraR, driving the protein to the dimer form. The dimer form, being more soluble, accumulates in the cytoplasmic compartment where it can productively interact with cognate promoters as defined by the presence of the *tra* box inverted repeat sequence.

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