

# A regulatory RNA from Ti plasmid coordinates expression of chromosomal operons involved in motility and attachment

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RNA regulators are generally small, untranslated and highly structured molecules. They regulate their targets generally through complementary base pairing with messenger RNAs (mRNAs) and affect directly or indirectly translation and/or stability of these mRNAs. They are frequently described as involved in cellular response to environmental transitions or stress (1). These conditions are usually found during interactions with a host, especially during pathogen interactions as in *Agrobacterium*. Nowadays importance of regulatory RNAs in regulation is largely admitted, whereas characterization of their biological functions and molecular mechanisms still remains a challenge.

In the reference strain *Agrobacterium fabrum* C58, many potential regulatory RNA have been identified (2,3 and 4). Among them, only three were functionally characterized and described in relation with plant-pathogen interaction: RepE, controlling replication of Ti plasmid (5); AbcR1, regulating transport of GABA (6) and RNA1111 involved in virulence of *A. fabrum*(4).

Within the team, we elaborated an original strategy that allowed us to functionally characterize regulatory RNAs. Using that, we characterized a novel RNA localized on Ti plasmid (RNA1083). We demonstrated that RNA1083 regulate ten genes by base pairing. Remarkably, these genes are clustered in three different operons and these three operons are localized in three different replicons: the two chromosomes and the Ti plasmid. Based on gene expression data and physiological analyses we have revealed the implication of RNA1083 in coordinate regulation of motility and attachment.

Here, we describe the first RNA regulator localized on plasmid and controlling chromosomal genes. Finally, the presence of this regulatory RNA on Ti plasmid –an accessory plasmid of *Agrobacterium* – supports the phenomenon of coevolution between plasmid and bacterium as well as the concept of "selfish" plasmid (7).

(1) Gottesman S and Storz G (2011). Bacterial Small RNA Regulators: Versatile Roles and Rapidly Evolving Variations. *Cold Spring Harb Perspect Biol.* 3(12):a003798-a003798.

(2) Wilms I, Overlöper A, Nowrousian M, Sharma CM, Narberhaus F (2012). Deep sequencing uncovers numerous small RNAs on all four replicons of the plant pathogen *Agrobacterium tumefaciens*. *RNA Biol.* 9(4):446-457.

(3) Lee K, Huang X, Yang C, Lee D, Ho V, Nobuta K, Fan JB, Wang K (2013). A Genome-Wide Survey of Highly Expressed Non-Coding RNAs and Biological Validation of Selected Candidates in *Agrobacterium tumefaciens*. *PLoS One.* 8(8):e70720.

(4) Dequivre M, Diel B, Villard C, Sismeiro O, Durot M, Coppée J.Y, Nesme X, Vial L, Hommais F (2015). Small RNA Deep-Sequencing Analyses Reveal a New Regulator of Virulence in *Agrobacterium fabrum*C58. *Mol Plant Microbe Interact.* 28(5):580-589.

(5) Chai Y, Winans SC (2005). A small antisense RNA downregulates expression of an essential replicase protein of an *Agrobacterium tumefaciens*Ti plasmid. *Mol Mic.* 56(6):1574-1585.

(6) Wilms I, Voss B, Hess WR, Leichert LI, Narberhaus F (2011). Small RNA-mediated control of the *Agrobacterium tumefaciens* GABA binding protein. *Mol Mic.* 80(2):492-506.

(7) Werren JH (2011). Selfish genetic elements, genetic conflict, and evolutionary innovation. *PNAS.* 108 Suppl 2:10863-10870.