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Review

Epigenetic control of *Agrobacterium* T-DNA integration ☆

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ABSTRACT

To genetically transform plants, *Agrobacterium* transfers its T-DNA into the host cell and integrates it into the plant genome, resulting in neoplastic growths. Over the past 2 decades, a great deal has been learned about the molecular mechanism by which *Agrobacterium* produces T-DNA and transports it into the host nucleus. However, T-DNA integration, which is the limiting, hence, the most critical step of the transformation process, largely remains an enigma. Increasing evidence suggests that *Agrobacterium* utilizes the host DNA repair machinery to facilitate T-DNA integration. Meanwhile, it is well known that chromatin modifications, including the phosphorylation of histone H2AX, play an important role in DNA repair. Thus, by implication, such epigenetic codes in chromatin may also have a considerable impact on T-DNA integration, although the direct evidence to demonstrate this hypothesis is still lacking. In this review, we summarize the recent advances in our understanding of *Agrobacterium* T-DNA integration and discuss the potential link between this process and the epigenetic information in the host chromatin. This article is part of a Special Issue entitled: Epigenetic Control of cellular and developmental processes in plants.

Contents

1. Introduction

Agrobacterium-mediated genetic transformation of plants is the only known natural example of trans-kingdom gene transfer. During transformation, *Agrobacterium* exports a single-stranded copy of the bacterial transferred DNA (T-DNA) into the host cell and ultimately integrates it into the host genome. In nature, *Agrobacterium* (*A. tumefaciens*) infects plant wounded tissues and causes neoplastic growths called crown gall tumors. In addition, under laboratory conditions, this phytopathogen has the ability to transform virtually any eukaryotic species, from fungal to human cells (reviewed in [1]). This unique feature distinguishes *Agrobacterium* as a versatile and powerful tool for molecular genetic studies as well as for plant biotechnology.

The *Agrobacterium* transformation process is coordinately regulated by the bacterial proteins and the host factors (for recent reviews, see [2–5]). Upon perception of plant phenolic compounds exuded from wound sites, *Agrobacterium* activates expression of several effectors, termed virulence (Vir) proteins, via the two-component (VirA-VirG) signal transduction system. Among the induced Vir proteins, VirD1 and VirD2 function together as an endonuclease complex and generate a single-stranded copy of T-DNA (T-strand) from a specific DNA segment that is defined by two border sequences of 25-bp direct repeats in the tumor-inducing (Ti) plasmid. Subsequently, the T-strand, with one VirD2 molecule covalently attached to its 5′ end (Fig. 1A), is exported into the

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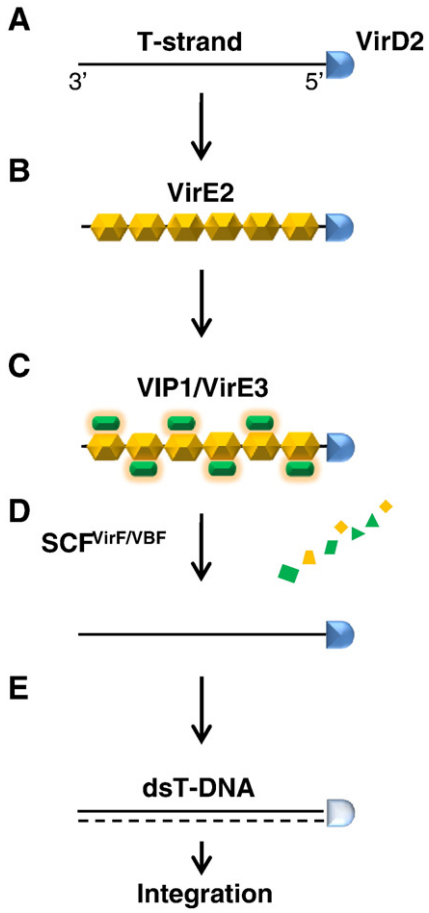

Fig. 1. Schematic overview of the T-complex formation and uncoating. (A) The *Agrobacterium* protein VirD2 is covalently attached to the 5′ end of the single-stranded (ss) T-DNA (T-strand) within the bacterial cell. (B) Numerous VirE2 molecules, which are most likely to be exported into the host cell independently of the T-DNA, directly bind to the T-strand, forming the T-complex. (C) In addition, the plant factor VIP1 (VirE2-interacting protein 1) and/or the *Agrobacterium* effector VirE3 interact with VirE2, facilitating the nuclear import of the T-complex. (D) Once the T-complex reaches the host cell nucleus, VIP1 and VirE2 are presumably removed from the T-strand by the *Agrobacterium* effector VirF and/or the plant factor VBF (VIP1-binding F-box protein). Both VirF and VBF are F-box proteins that function in the SCF (Skp1-Cul1-F-box protein) ubiquitin E3 ligase complex (SCF^{VirF} and SCF^{VBF}, respectively) and target VIP1 as well as its associated protein VirE2 for proteasome-dependent degradation. It remains elusive whether and how VirE3 and VirD2 dissociate from the T-strand. (E) The T-strand is likely to be converted into a double-stranded form (dsT-DNA) before T-DNA expression and/or integration. Whether VirD2 is still attached to the T-strand during this conversion is also unknown.

host cell through a type IV secretion system (T4SS) composed of the VirB and VirD4 proteins. Moreover, with the help of their C-terminal export signals [6], at least four other bacterial effectors (VirD5, VirE2, VirE3, and VirF) are also translocated into the host cell through the T4SS channel [6,7], facilitating the rest of the transformation process.

Within the host cytoplasm, the T-DNA is believed to exist as a nucleoprotein complex (T-complex), in which it is coated with numerous VirE2 molecules (Fig. 1B; [8]). Furthermore, the plant factor VIP1 (VirE2-interacting protein 1), which contains a functional nuclear localization signal (NLS), interacts with VirE2 (Fig. 1C) and facilitates the nuclear import of T-DNA [9]. To augment this VIP1 function, *Agrobacterium* exports into the host cell another bacterial effector VirE3 [10]; like VIP1, the VirE3 protein also possesses functional NLSs and mediates the T-DNA nuclear import via its direct binding to VirE2 (Fig. 1C; [10]).

After the T-complex enters the cell nucleus, the coating proteins are most likely removed from the T-strand by the VirF-mediated protein degradation (Fig. 1D; [11]). VirF, the first F-box protein identified in prokaryotes [12], functions as a subunit of the SCF (Skp1-Cul1-F-box protein) ubiquitin E3 ligase complex in the host cell and targets VIP1 as well as its associated protein VirE2 for proteasome-dependent degradation [11]. In addition, the plant F-box factor VBF (VIP1-binding F-box protein) is involved in the T-complex uncoating in a manner similar to VirF (Fig. 1D; [13]). The finding that VirE3 and VirF bacterial effectors possess functional host analogs, VIP1 and VBF, respectively, indicates potential convergent evolution [14] and underscores the importance of the transformation steps mediated by these factors for the infection process. Furthermore, VIP1 and VBF are components of the plant defense system [13,15,16], indicating the ability of *Agrobacterium* to subvert the host defense machinery for active promotion of infection.

The T-complex proteasomal uncoating process is likely to be a prerequisite for conversion of the T-strand into the double-stranded DNA (dsT-DNA) and its subsequent expression and/or integration into the host genome (Fig. 1E). However, potentially in a defense response of the host plant, VirF is rapidly degraded via the host ubiquitin/proteasome pathway, and *Agrobacterium* has evolved another exported effector, VirD5, to interact directly with and stabilize the VirF protein (Magori S. and Citovsky V., unpublished).

The entire process of *Agrobacterium*-mediated genetic transformation is reminiscent of the retrovirus-mediated gene transfer. However, unlike retroviruses, *Agrobacterium* does not export any proteins that function as an integrase. Moreover, none of the known exported bacterial effectors has been clearly demonstrated to play a direct role in T-DNA integration. Therefore, *Agrobacterium* most likely exploits the host factors to complete this process. In recent years, the host DNA double-strand break (DSB) repair has received increasing attention as a primary mechanism that facilitates T-DNA integration [17]. In this review, we focus on the potential role of the DSB repair machinery in *Agrobacterium* genetic transformation and also discuss how chromatin dynamics affects DSB repair and, by implication, T-DNA integration.

2. DSB represents the primary target site of T-DNA integration

As an indirect means to dissect the molecular mechanism underlying T-DNA integration, it is important to understand where in the host genome T-DNA is ultimately targeted. Large-scale analyses of T-DNA insertion distribution patterns in *Arabidopsis* suggest that the integration occurs preferentially in gene-rich euchromatic regions of the plant genome [18–20]. However, all these analyses were done using transgenic plants that had been positively selected based on the marker gene expression. Thus, the seemingly non-random integration pattern may be just a consequence of the variable transcription activity at the initial integration sites. To address this problem, a more recent work utilized *Agrobacterium*-transformed plant cells propagated under non-selective conditions and found a high frequency of T-DNA insertions even in the heterochromatic regions [21]. Furthermore, the integration pattern did not correlate with the genomic DNA methylation pattern [21]. Together, these observations suggest that T-DNA integration per se takes place randomly throughout the genome, regardless of the DNA sequences or the transcription activity at the pre-integration sites [21].

Given that T-DNA integration is truly random, what could be the limiting factor of this event? Several lines of evidence suggest that T-DNA integration may depend on the availability of naturally occurring DNA double-strand breaks (DSBs) in the host genome. Indeed, exposure of plants to DSB-inducing agents, such as X-rays, is known to enhance integration of foreign genes [22]. In addition, it has been shown that induction of DSBs by transient expression of a rare-cutting restriction enzyme in plant genomes increases the T-DNA integration frequency [23–25]. Thus, *Agrobacterium* likely utilizes DSBs as the primary target sites of T-DNA integration. However, the possibility that other DNA lesions, such as single-strand breaks, may also serve as the potential integration sites cannot be excluded.