Mini-Review

Agrobacterium aiming for the host chromatin

Host and bacterial proteins involved in interactions between T-DNA and plant nucleosomes

Benoît Lacroix* and Vitaly Citovsky

Department of Biochemistry and Cell Biology; State University of New York; Stony Brook, New York USA

Abbreviations: T-DNA, transferred DNA; VIP1, VirE2-interacting protein 1; ssDNA, single strand DNA; NHEJ, non homologous end joining

Key words: histones, nucleosomes, VirE2-interacting protein 1 (VIP1), VirE2, chromatin targeting, T-complex

Agrobacterium genetically transforms its hosts by transferring a segment of DNA (T-DNA) into the host cell and integrating it into the host genome. Integration requires a close interaction between T-DNA, which is packaged into a nucleoprotein complex (T-complex) by bacterial virulence (Vir) proteins, and the host chromatin. This interaction is facilitated by the host protein VIP1, which binds both to the major protein component of the T-complex, VirE2, and to the core histones. Recently, VIP1 has been demonstrated to mediate the interaction between plant nucleosomes and VirE2-DNA complexes (i.e., synthetic T-complex-like structures) in vitro. Here, we discuss major implications of these observations—such as the possible role of core histone modifications, proteasomal uncoating of the T-complex mediated by the bacterial F-box protein VirF, and the need for changes in chromatin structure to render it accessible to the T-DNA integration—for the process of chromatin targeting of foreign DNA and its integration into the eukaryotic genome.

Introduction

Agrobacterium tumefaciens is a soil phytopathogen with a unique ability to transfer a segment of its DNA (T-DNA) into the genome of eukaryotic cells. Although plants are the natural hosts for Agrobacterium, this microorganism can also genetically transform a wide range of other eukaryotic species, from fungi1,2 to human cells.3 Expression of the T-DNA genes in host plant cells results in uncontrolled cell proliferation due to modification of growth regulator balance and synthesis of opines, molecules that can be used by the bacteria as a source of carbon and nitrogen (reviewed in ref. 4). T-DNA is transported from the bacterium to the host cell as a nucleoprotein complex (T-complex) with bacterial virulence (Vir) proteins as well as host proteins. Briefly, one molecule of VirD2 is covalently attached at the 5’ end of the T-strand, and numerous VirE2 molecules coat the T-strand over its entire length.12 Nuclear import of the T-complex is mediated by VirD2, which binds directly to the host importin α,13 and by VirE2, which does not bind importin α efficiently,14 but interacts with VIP1 (VirE2 interacting protein 1)15 which then binds importin α, acting as a molecular adaptor between VirE2 and the importin α-dependent nuclear import machinery.14,16

While the processes of the T-strand formation, its export to the host cell, and nuclear import of the T-complex are relatively well studied, the mechanism by which the T-complex is targeted to and associates with the host cell chromatin remains enigmatic. The cell nucleus is a complex organelle within which macromolecular traffic is tightly regulated.17,18 It is thus likely that a specific mechanism exists to target a large structure, such as the T-complex, to the chromatin. To gain an insight into this mechanism, we explored in vitro interactions between plant nucleosomes, VIP1 and other components of the T-complex.19

Requirement for a Link Between the Host Chromatin and the Invading T-Complex

Integration of the T-DNA into host genome obviously requires a close interaction with the chromatin. The ability of VIP1 to bind core histones,20,21 and interact with VirE2 and, therefore, the T-complex,15 makes it an attractive candidate for this role of a molecular link between the T-complex and the host chromatin. Our results19 indeed demonstrate that VIP1, besides binding strongly and specifically to nucleosomes, is able to mediate in vitro interaction between nucleosomes and VirE2 as well as between nucleosomes and VirE2-ssDNA complexes, i.e., synthetic T-complex-like structures (Fig. 1). These data are consistent with the demonstrated role of VIP1 in planta as a specific enhancer of the Agrobacterium-mediated gene transfer.16 In respect to its roles in Agrobacterium infection, VIP1 contains two functional domains: an N-terminal domain that

*Correspondence to: Benoît Lacroix; Department of Biochemistry and Cell Biology; State University of New York; Stony Brook, New York 11794-5215 USA; Tel.: 631.632.1015; Fax: 631.632.8575; Email: blacroix@notes.cc.sunysb.edu
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interacts with VirE2 and a C-terminal domain that binds core histones in vivo. Similarly, in vitro, the N-terminal domain of VIP1, while still able to bind VirE2, does not mediate the nucleosome-VirE2 interaction. Collectively, these observations establish the role of VIP1 as a molecular bridge between nucleosomes and VirE2. However, the role in chromatin targeting of additional plant factors interacting with the T-complex—e.g., VirD2-binding proteins or another VirE2-binding protein, VIP2—cannot be ruled out. VIP1, therefore, may be only one of a series of factors that can mediate the T-complex interaction with the host chromatin prior to and during the T-DNA integration.

**Is There a Role for Histone Modifications During the T-Complex Chromatin Targeting?**

We did not detect preferential binding to VIP1 to modified histones (acetylated histone H3 and lysine 4-methylated histone H3) which represents markers of transcriptionally active chromatin regions. This is consistent with recent observations that T-DNA integrates randomly, irrespective of the transcriptional state of the chromatin. However, other types of histone covalent modifications might be involved in targeting the T-complex to the integration sites. For example, the T-DNA integration is thought to occur preferentially at double-stranded DNA breaks (DSBs), and non-homologous end joining (NHEJ) machinery is involved in the integration process.

A site-specific phosphorylation at serine-139 of histone H2A, which specifically occurs in the DSB regions, is necessary for recruitment and binding of the NHEJ proteins. In animals and yeast, this phosphorylation involves a specific variant of H2A, H2AX, whereas, in plants, the corresponding H2A variants, termed HTA3 and HTA5, were also shown to undergo the serine-139 phosphorylation. It is tempting to speculate that phosphorylated HTA3 and HTA5 may bind a component of the T-complex, such as VIP1, with a higher affinity than their non-phosphorylated forms, thereby “attracting” the T-complex to the DSB sites. Our initial experiments using HTA3 and its phosphorylation-mimicking mutant S139D, did not detect such preferential binding of VIP1 (Lacroix B and Citovsky V, unpublished). However, the negatively charged amino acid substitution may not have faithfully reproduced the full spectrum of the effects of HTA3 phosphorylation on chromatin at the DSB site, or other protein factors may be required for modulation of VIP1 binding.

**When Does the T-Complex Uncoating Occur?**

Before integration can take place, the proteins coating the T-strand must be removed. Our earlier data indicate that this is achieved by proteasomal degradation of VirE2 and VIP1 and this degradation is mediated by a bacterial F-box protein, VirF, which is translocated into host cell and recognizes VIP1 as a substrate for the SCFVirF complex. Yet, it remained unknown whether VirF can recognize the T-complex while it is already associated with the host chromatin. Our new data indicate that VirF can bind VIP1 when the latter is still attached both to nucleosomes and VirE2-ssDNA complexes. Thus, VirF most likely functions at the stage when the T-complex is bound to the chromatin via VIP1.

But is there a mechanism to prevent “premature” uncoating that could occur before chromatin targeting? Potentially, additional host proteins or bacterial virulence proteins exported to the host cell may prevent VirF binding to VIP1 and/or to the ASK1 component of the SCFVirF complex, until the T-complex is in contact with chromatin. Moreover, because T-DNA can be integrated as double-stranded DNA, removal of the coating proteins may be necessary before the second-strand synthesis can occur. It is still unknown whether the second-strand synthesis occurs before or after the T-complex associates with the chromatin. Our results suggest that the T-complex first interacts with the chromatin via VIP1, then the T-complex is uncoated via the SCFVirF pathway, exposing the T-strand for the second-strand synthesis and integration, which may represent coupled events.

**Chromatin Form “Susceptible” for the T-DNA Integration**

Another interesting aspect of the T-DNA integration process is that not only the T-strand must be uncoated of its associated proteins, but also the target host DNA must be made accessible to the integration machinery, potentially by decondensing, or even partially unpacking the chromatin. Although earlier data suggested that T-DNA integrates mainly into active (decondensed) regions of the chromatin, more recent evidence indicates that the integration is truly random and independent of active or inactive state of the target chromatin. Yet, even if the T-DNA can integrate equally well into both euchromatin and heterochromatin, at least a local and transient decondensation of the chromatin is most likely required for efficient integration. While little is known about how the host chromatin is perturbed during the T-DNA integration, several potential scenarios can be envisioned.

The T-complex can be targeted preferentially to the chromatin regions, which are already present in a form “susceptible” to integration. For example, DSBs, into which the T-DNA preferentially integrates and in which the DNA may be already exposed to the repair machinery, may represent such sites. Alternatively, a change of the chromatin structure may be transiently induced by one of the protein components of the T-complex itself during its interaction with chromatin. For example, VirF can bind VIP1 while VIP1 is attached both to nucleosomes and to the VirE2-ssDNA complex (see Fig. 1). If, under these conditions, VirF promotes degradation of...
VirE2 attached to VIP1, it may, in the same manner, induce degradation of the core histones which are also bound to VIP1.\(^{19,21}\) Such local destabilization of the core histones would create a chromatin region favorable to the T-DNA integration.

**Conclusions**

Increasing evidence indicates that Agrobacterium has evolved to subvert many diverse cellular systems for its own needs.\(^7,38\) One of the best examples of this strategy is the reliance of Agrobacterium on the host VIP1 protein. While the endogenous function of VIP1 is still under investigation, its role in chromatin remodeling and transcriptional regulation is well established.\(^{38,39}\) For example, Agrobacterium tumefaciens encodes a VirE3 protein that is imported into the host cell's nucleus and, at least partially, mimics the VIP1 function.\(^{44}\) In addition, some strains of Agrobacterium rhizogenes do not encode VirE2, but instead use the GALLS protein, which fulfills the VirE2 functions and likely uses a VirE2-independent pathways for nuclear import, interaction with chromatin, and integration.\(^{45}\) Overall, while Agrobacterium utilizes its own molecular systems for introduction of DNA and proteins into the host cells, it increasingly exploits the host factors for subsequent stages of genetic transformation, especially those, such as chromatin targeting and uncoating of the T-complex and subsequent T-DNA integration, that take place in the host cell nucleus.

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