CHAPTER 6

Nuclear Import of *Agrobacterium* T-DNA

Tzvi Tzfira, Benoit Lacroix and Vitaly Citovsky

Agrobacterium-mediated genetic transformation is a process by which genetic material is transported from the bacterium into the host nucleus, where it stably integrates. The transferred DNA (T-DNA) is escorted, by two bacterial proteins, as a single-stranded DNA-protein complex (a T-complex), which mediate its transport to the host nucleus. The large size and mass of this DNA-protein complex raise questions as to the molecular machinery and mechanism by which the T-complex passes the nuclear pore barrier. Recent studies have revealed the important role of specific host proteins in interacting with and guiding the T-complex through the nuclear pore, and to its point of integration. In this chapter, we summarize our knowledge of the function of T-DNA bacterial and host protein chaperones, and draw a model for their action during the nuclear import and intranuclear transport of *Agrobacterium* T-DNA.

Introduction

Agrobacterium-mediated genetic transformation of plant cells is a unique and complicated process by which genetic material is transported from the bacterium into the host nucleus, where it stably integrates (see Fig. 1 and reviews in refs. 1-5). Although in nature, *Agrobacterium* transforms mainly dicotyledonous plants,[6] under controlled culture conditions it has been shown to possess a broader host range which includes monocot plants, yeast[7] and other fungi,[8,9] and even human cells.[10] In modern plant breeding, *Agrobacterium* is widely used for plant genetic engineering.[11] The molecular basis for the transformation process has therefore been the subject of numerous studies over the past several decades (reviewed in refs. 1-5).

Agrobacterium tumefaciens is a gram-negative bacterium which is the causative agent of crown-gall disease in many dicotyledonous plant species.[6] The disease symptoms result from the transfer, integration and expression of a specific DNA fragment (known as transferred DNA or T-DNA) from the bacterial tumor-inducing (Ti) plasmid into the plant cell genome. The machinery needed for the generation of transferred T-DNA and for its transport into the host cell is encoded by a serie of chromosomal (*chv*) and Ti-plasmid-encoded virulence (*vir*) genes (reviewed in refs. 1-5). Interestingly, although the bacterial proteins possess many of the functions needed for the transformation process, host-plant factors also play a crucial role in the T-DNA nuclear import and integration (reviewed in refs. 2-4). In this chapter, we discuss the role of both *Agrobacterium* and host proteins during *Agrobacterium* T-DNA long voyage in the host cell, from its cytoplasmic point of entry to the nucleus.

Nuclear Import and Export in Plants and Animals, edited by Tzvi Tzfira and Vitaly Citovsky.

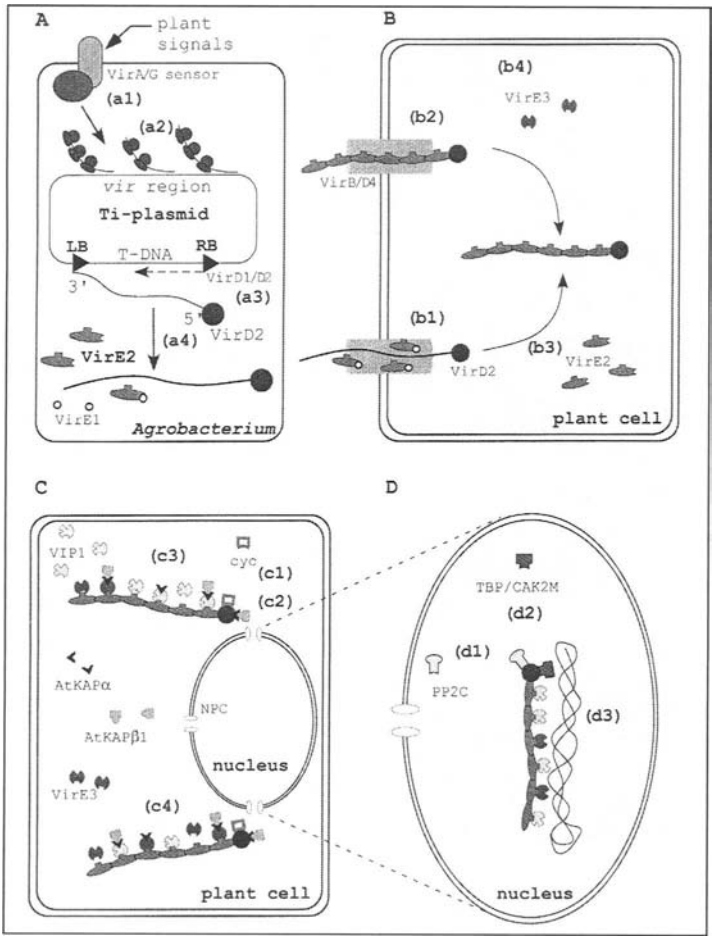

Figure 1. The process of *Agrobacterium*-mediated genetic transformation and model for the nuclear import of the *Agrobacterium* T-complex. A) Induction of the *Agrobacterium* virulence (*vir*) genes and production of the T-complex. The transformation process begins with sensing of plant signals by the *Agrobacterium* VirA/VirG two-component sensory machinery (a1) and transcriptional activation of the *vir* region (a2). The T-DNA left and right borders (LB and RB, respectively) are nicked by the VirD2/VirD1 endonuclease complex, and a single stranded T-DNA copy (T-strand) is released from the Ti plasmid (a3) to produce an immature T-complex composed of the T-strand and a single VirD2 molecule attached to its 5'-end (a4). B) Export into the host cell cytoplasm and assembly of the mature T-complex. Immature (b1) or mature (b2) T-complexes are exported into the host cell cytoplasm through the VirB/VirD4 channel. If exported individually, the immature T-complex and VirE2 molecules are later assembled into a mature T-complex in the host cell cytoplasm (b3). The VirE3 (b4) protein is also exported into the host cell cytoplasm through the same VirB2/VirD4 channel and functions later in nuclear import of the mature T-complex. C) Nuclear import of the mature T-complex. While traveling toward the nuclear pore, VirD2 interacts with cyclophilins (c1), which may maintain its proper conformation in the cytoplasm. For their nuclear import, VirD2 and VirE2 employ two different mechanisms: VirD2 is imported directly by AtKAPα (c2), while VirE2 is imported by VIP1 (c3) or VirE3 (c4) which function as molecular adaptors between VirE2 and the AtKAPα-dependent nuclear import pathway. D). Intranuclear transport to the site of integration. Once inside the nucleus, VirD2 may undergo dephosphorylation by PP2C (d1). Interaction of VirD2 with CAK2M and TBP (d2) and VirE2 with VIP1 (d3), all presumed members of the host cell transcriptional complexes, may result in the intranuclear transport of the T-complex to the point of integration in the plant chromatin.