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Chromatin proteins and their role in plant genetic transformation

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Abstract

Agrobacterium-mediated plant transformation is one of the most widely used technologies to introduce gene in plants. Plant-microbe interaction has been extensively studied in the last decade and several factors involved in this complex process such as nuclear import, T-DNA integration and expression have been identified. Despite a large amount of data available with regards to the bacterial factors involved in transformation, there are little known about host plant factors involved in this process. Moreover, the host plant responds to several cellular processes and the chromatin alteration is linked with the foreign DNA integration and finally with gene expression. Here, the current knowledge on the role of chromatin proteins during Agrobacterium-mediated plant transformation is briefly described.

Keywords: chaperons, Agrobacterium mediated transformation, T-DNA integration, gene expression

Introduction

Plant genetic transformation: a complex process of bacteria-plant interaction

During the past decade, the concept of using *Agrobacterium tumefaciens* as a vector to create transgenic plants was viewed as a prospect and a "wish" [25]. Today, many important species are routinely transformed using this bacterium, and the list of species that are susceptible to *Agrobacterium*-mediated transformation seems to grow daily [4, 24, 25, 26, 31, 34]. In some developed countries, a high percentage of economically important crops are genetic modified and an increasing number of these transgenic plants are or will soon be generated by *Agrobacterium*-mediated transformation. There still remain, however, many challenges for genotype-independent transformation of many economically important crop species, as well as forest species. In addition, predictable and stable expression of transgenes remains problematic [1, 27].

Agrobacterium tumefaciens transfers a segment of its DNA (T-DNA) to plant cells, where it integrates into the plant genome. In higher eukaryotic systems, the predominant mechanism of integration of naked DNA (single stranded DNA, ssDNA) molecule is illegitimate recombination [7, 28]. T-DNA integration can occur at random in any chromosome and likely involves illegitimate recombination of the T-DNA ends with a plant target sequence. On the other hand, one plant cell can contain several T-DNA molecules that can be present at one locus or at several independent loci [21]. These loci frequently consist of several T-DNA copies linked in an inverted or direct orientation. Different mechanisms could account for the formation of these repeat structures. For example, in the replication model it was postulated that the repeats would originate from a single T-DNA by replication and repair before or during insertion into the plant DNA [9, 10]. In the other hand, in the ligation model, repeat structures would originate from the ligation of two double-stranded T-DNA copies prior to or during integration into the plant genome. Nonetheless, it is desirable to develop methods that can generate a high percentage of transgenic plants capable of expressing the transgene consistently over successive generations. The precise integration of a transgene into a predetermined genomic location can reduce variation in transgene expression [36].

In the past several years, the T-DNA integration research has shifted from analyzing integration junction sequences to identifying the molecules that are involved in the integration process [2, 37]. Using yeast and *Arabidopsis* as models, several host genes involved in T-DNA integration have been identified, although many others likely await discovery. Research in this area will lead not only to the identification of the proteins involved in the integration process but also to elucidation of the precise molecular mechanism resulting in successful integration. In addition, understanding the molecular pathway(s) of T-DNA integration will lead to the development of new tools and approaches for controlling this process. Such tools might then be used for efficient gene-targeting and gene-replacement strategies, which are highly valuable for plant research and biotechnology.

Tremendous data are now available with regards to identification of genes and proteins involved in integration. Previous studies indicated that T-DNA preferentially integrates into transcriptionally active regions of the genome, especially in 5′-promoter regions. This would make sense, considering that chromatin structure surrounding active promoters may be more "open" and accessible to foreign DNA. However, recent results suggest that this seemingly non-random pattern of integration may be an artifact of selection bias, and that T-DNA may integrate more randomly than previously thought [25]. Nevertheless, it is not known yet, how T-DNA molecule sounded by VirE2 proteins and VirD2 protein at the 5' region, is integrated in plant chromatin [6]. It was suggested that VirD2 can recruit several plant proteins such ligase and help to integration of T-DNA. VirE2 protected T-DNA from nucleolytic degradation and by interaction with host protein VIP1 may form a link between T-DNA and plant chromatin [29, 30]. VIP1 mediated the interaction between plant nucleosomes and VirE2 complexes *in vitro* [8].

Recently, it has been shown that proteins from *vir* operon, located outside of T-DNA region are important for translocation and T-DNA processing. One of these proteins, VirC2, a 202aa cytoplasm protein from *A. tumefaciens*, enhanced T-strand transfer and virulence through its C-terminal ribbon-helix-helix DNA binding fold [15]. Vir C2 function in T-DNA processing resembles the function of family of DNA binding proteins accessory to endonucleases for ssDNA processing during bacterial conjugation [32]. However, plant proteins play important role in T-DNA targeting by interaction with other chromatin factors that can mediate the T-complex integration prior or during T-DNA integration.

Host chromatin proteins play important role in transformation process

Although chromatin structure plays important role in regulation of endogenous gene and silencing invasive foreign DNA, there are evidences that chromatin proteins participates in foreign integration events.

Interaction between *Agrobacterium* and plant required the participation of both bacteria and plant factors. Recently, it has been shown that chromatin factors are important candidates for improving *Agrobacterium*-mediated plant transformation [11, 27]. Plants may respond to *Agrobacterium* infection and this response may involve differential plant gene expression. Genes that are induced or repressed during the early stages of *Agrobacterium*-mediated transformation may provide targets for manipulation of the host to improve the efficiency of transformation of recalcitrant plant species. Therefore, involvement of many of these chromatin genes in *Agrobacterium*-mediated transformation has not yet been rigorously established.

However, a laborious screening of T-DNA tagged mutants was conducted with regards to determination of their potential role in transformation and it has been suggested that several some plant proteins play important role in integration. For example, Nam et al (1997) showed that some proteins, variety specific, are very important in transformation [16]. The authors showed that the *rat* phenotype of the radiation-hypersensitive *Arabidopsis* ecotype UE-1 results from a deficiency in T-DNA integration. Another study showed the

some of histone protein HTA1 (H2A1) are important for T-DNA integration [17, 18]. An *Arabidopsis* mutant (*rat5*) containing a T-DNA insertion in the 3'-UTR of the histone *H2A1* gene shows a large reduction in stable but not transient transformation efficiency by *Agrobacterium* [17, 19]. Other *Arabidopsis rat* mutants, identified following T-DNA mutagenesis or RNAi expression, likely disrupt expression of genes involved in chromatin structure and remodeling such as histones, histone acetyltransferases, and histone deacetylases [38]. Some of these mutants show high transient but low stable transformation efficiency, suggesting that the *rat* phenotype most likely results from inhibition of T-DNA integration. Recently, the over-expression of several histone genes has been analyzed in *Arabidopsis* and it has been shown that some histone, H2A and H2B but not H3 and H4, increases *Agrobacterium* mediated transformation [11, 23]. The authors suggested that certain histones enhance transgene expression, protect incoming transgene DNA during the initial stages of transformation, and subsequently increase the efficiency of *Agrobacterium*-mediated transformation.

Moreover, several additional *Arabidopsis rat* mutants contain T-DNA insertions have been targeted by RNAi. These include lines with disruptions in expression of receptor-like protein kinases, transcription factors, expansins, and chromatin-remodelling factors [38]. Although characterization of the roles of these proteins in the transformation process will require intensive investigation, the diversity of the genes identified so far suggests a significant plant contribution to the *Agrobacterium*-mediated transformation process.

On the other hand, besides the role of chromatin factors, several other factors play crucial in T-DNA integration such as factors involved in repair and recombination. As we already mention, the integration of T-DNA in host genome is via illegitimate recombination, which does not required higher homology between T-DNA and target sites. Once DNA is integrated the double stranded break must be repaired. However, in plants several factors are involved in repair such as Ku70, Ku80, Mre11/Rad50/Nbs1 complex, XRCC4 and Lig4. Alternatively, DSB can be also repaired by factors such as RPA, Rad51. Only few of these factors were studied for their potential role in T-DNA integration. For example, Ziemienowicz et al. (2000) suggested that a plant-specific ligase may be involved in the T-DNA integration [5]. Whereas one study suggested the involvement of DNA ligase IV in T-DNA integration [13], two other studies indicated that ligase IV was not involved in this process [12]. It is not clear why the results were contradictory. Bakó et al. (2003) reported that VirD2 protein is phosphorylated in plants by a nuclear kinase and associates tightly with the TATA box-binding protein (TBP) [20]. The authors suggested that because TBPs are involved in transcription-coupled repair, they may have a role in T-DNA integration. Mutation of *Ku* genes results in hypersensitivity to DNA-damaging agents (irradiation, bleomycin). For example, an *Arabidopsis Ku80* mutant showed a reduction in transformation efficiency (*rat* phenotype) and over-expression of *Ku80* showed an increase in susceptibility to transformation [14]. However, the role of this factor is still ambiguous. Despite the rapid advance in this area, described by the tremendous number of papers, it is evident that we are just at the beginning of understanding the molecular mechanism of chromatin in plant transformation.

Histone chaperones and T-DNA integration

Several indications suggested that chromatin chaperone play important role in T-DNA integration. Histone chaperones are known as a family of proteins that facilitate histone deposition and allow histone exchange and eviction during nucleosome assembly and disassembly [3]. They are involved in the histone storage, histone translocation to the nucleus, and histone exchange and histone deposition onto the DNA for replication dependent chromatin assembly. Several article described different category of histone chaperone which are involved in replication, DNA repair, in nucleosome assembly and disassembly etc. These histone chaperones are conserved in eukaryotes cells and they also have several functions.

In his way to plant chromatin, T-DNA which is wrapped with proteins is expected to recruit other proteins which will remove or remodel the chromatin. For example, T-DNA integration increases when chromatin assembly factor 1 (CAF1) is absent [22]. The authors showed that depletion of either subunit of CAF-1 (FAS1, FAS2 and MSI1 subunit) increased the frequency of somatic homologous recombination (HR) in planta with approximately 40-fold. The frequency of transferred DNA (T-DNA) integration was also elevated. A delay in loading histones onto newly replicated or repaired DNA might make these DNA stretches more accessible, both to repair enzymes and to foreign DNA.

As we mentioned above, the reduction of activity of several chromatin factor will have an effect T-DNA integration. Recently, Crane and Gelvin, 2007 showed that several chromatin genes are involved in transformation [39]. These genes comprises 15 gene family groups encoding for important proteins such as bromodomain proteins, chromodomain proteins, chromatin remodeling complexes, DNA methyltransferases, global transcription factors, histone acetyltransferases, histone deacetylases, histone H1, methyl binding domain proteins, MAR binding filament-like proteins, nucleosome assembly factors, SET domain proteins, silencing groups A, B, and F. Silencing of 24 chromatin genes reproducibly resulted in some level of decreased transformation susceptibility. In particular, silencing of the genes encoding the chromatin protein SGA1 (At5g38110) and histone deacetylases HDT2 (At5g22650) and (to a lesser extent) HDT1 (At3g44750) resulted in a *rat* phenotype [39]. To conduct these studies, three different standard transformation assays that measured crown gall tumorigenesis, resistance to an antibiotic or herbicide, and transient expression of GUS activity has been used. Because chromatin proteins may also be involved in transgene expression, most of the studies were concerned that in these *rat* mutants T-DNA may have integrated into the genome but may not have subsequently been expressed. Therefore, three RNAi *rat* mutants (SGA1, HDT1, and HDT2) which shown a *rat* phenotype, were analyzed for T-DNA integration into high molecular weight plant DNA in the absence of selection for transgene expression [27, 39].

The results of these experiments indicated that decreased expression of these three genes resulted in a severe reduction in T-DNA integration, similar to that observed in the *rat*5 (HTA1) mutant using the same non-selective integration assay [19]. We were also interested to know if over-expression of such factors, *sga1* and *hdt2* will have an effect in transformation or gene expression. However, when we over-expressed *sga1* cDNA in *Arabidopsis* plants and we tested several mutants for their susceptibility to transformation, we have observed an increase in transformation efficiency (figure 1). *Arabidopsis* mutant's over-expression *SGA1* showed a *hat* phenotype.

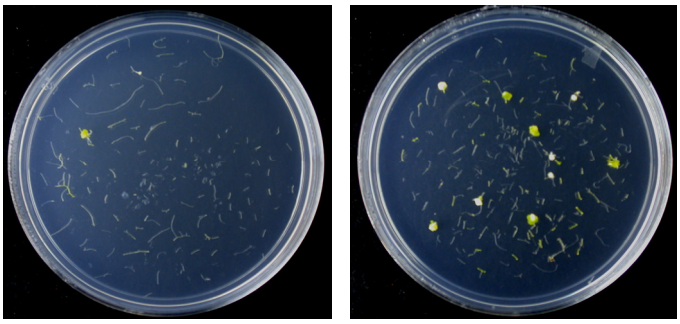

Figure 1. Tumors formation on wild-type (WS) and over-expression *sga1* line (SGA1-L13) after *Agrobacterium* mediated transformation (10^{-9}cfu/ml)

Another interesting aspect is that the over-expression of over-expression of a SGA1 cDNA, in contrary of other histones cDNAs, did not increase transient expression of a *gusA* gene co-transfected into tobacco BY-2 protoplasts (figure 2). Moreover, over-expression of

SGA1 did not increase expression of a previously integrated *gusA* transgene, (unpublished data). However, over-expression of this factor increases transformation through other mechanism than increasing genes expression.

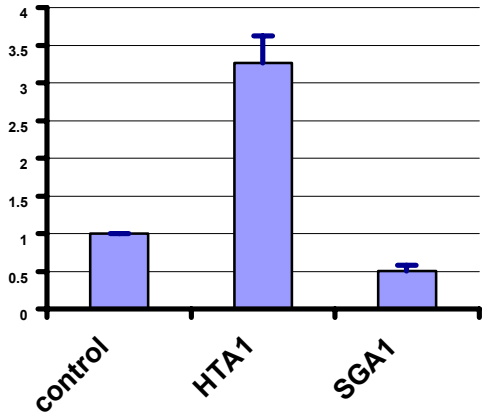

Figure 2. Over-expression of *HTA1* increases transient transgene expression, over-expression of *SGA1* decreases transgene expression.

Taken together, it has been demonstrated that several proteins respond to *Agrobacterium* mediated transformation. The responses are correlated with transgene expression or T-DNA integration. Actually the mechanism by which these factors influenced transformation is under evaluation. In most transformation events the effect of one or combination of two genes/proteins has been tested. However it is now known yet if those mutated factors or over-expressed once can have a positive or negative effect in transformation. These proteins may interact one with each other and, a strong canonical correlation with a specific combination of those genes/proteins illustrated the complexity of this transformation process.

Conclusions and perspectives

In the past 20 years tremendous progress has been made in plant transformation. Transgenic plants have gained extraordinary significance in fundamental research and applied genetics. By using forward and reverse genetics many chromatin-related factors have been discovered as been important in transformation process but nonetheless there are many aspects related with molecular mechanisms which need to be investigated in near future.

Therefore, there are still open questions regarding the proteins and genes involved in transformation, such as: 1) Why certain chromatin proteins influenced transformation and gene expression and other not?; 2) T-DNA integration process is controlled by certain histones and histone interacting proteins but how?; 3) How we can improve the transformation to induce de homologous recombination?; 4) Which are the optimal parameters for gene targeting activity?

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