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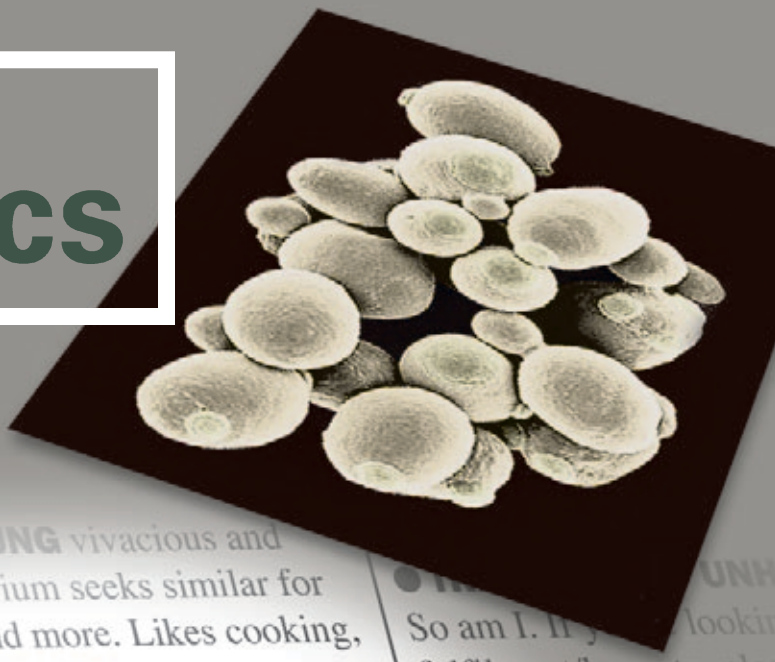

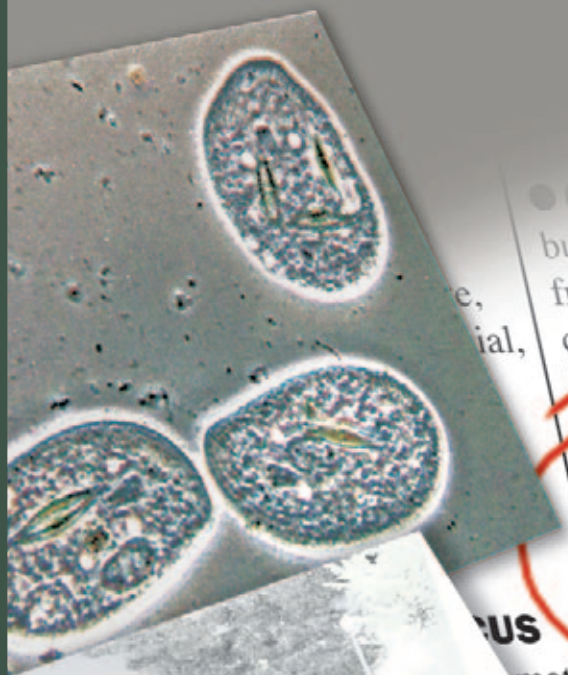

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Promiscuous *Agrobacteria* seek new partners

On a chromosome far, far away

Oncogenic translocations

Global synthetic lethality

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It's long been known that *Agrobacterium tumefaciens* can transfer its tumor-inducing (Ti) plasmid into plant genomes to cause crown gall disease. However, it's recently been shown that *Agrobacterium* can transfer its DNA to several species including yeast, fungi and human cells – ensuring a bright future for this bacterium in genetic engineering. On page 29–37 of this issue, Benoît Lacroix and colleagues discuss the challenges that the promiscuous *Agrobacterium* faces in its search for new 'partners'. Cover design by Geraldine Woods.

Review TRENDS in Genetics Vol.22 No.1 January 2006

A case of promiscuity: *Agrobacterium*'s endless hunt for new partners

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Agrobacterium tumefaciens **is a phytopathogenic bacterium that induces the 'crown gall' disease in plants by transfer and integration of a segment of its tumor-inducing (Ti) plasmid DNA into the genome of numerous plant species that represent most of the higher plant families. Recently, it has been shown that, under laboratory conditions, the host range of *Agrobacterium* can be extended to non-plant eukaryotic organisms. These include yeast, filamentous fungi, cultivated mushrooms and human cultured cells. In this article, we present *Agrobacterium*-mediated transformation of non-plant organisms as a source of new protocols for genetic transformation, as a unique tool for genomic studies (insertional mutagenesis or targeted DNA integration) and as a useful model system to study bacterium–host cell interactions. Moreover, better knowledge of the DNA-transfer mechanisms from bacteria to eukaryotic organisms can also help in understanding horizontal gene transfer – a driving force throughout biological evolution.**

Introduction

Almost 100 years after its discovery as the causative agent of crown gall disease [1], the Gram-negative soil phytopathogen *Agrobacterium tumefaciens* is still central to diverse fields of biological and biotechnological research, ranging from its use in plant genetic engineering [2] to representing a model system for studies of basic biological processes underlying genetic transformation [3,4]. The natural host range of *Agrobacterium* among species of the plant kingdom is rather extensive and includes members of most of the plant families. This had already been determined in the early 1970s by infecting various plant species with wild-type *A. tumefaciens* [5] or *Agrobacterium rhizogenes* [6] and monitoring for disease symptoms (i.e. galls or hairy roots induced by *A. tumefaciens* or *A. rhizogenes*, respectively). These pioneering studies revealed that, although none of the *Bryophytae* (e.g. mosses) and *Pteridophytae* (e.g. ferns) species could be transformed by *Agrobacterium*, up to 56% of the gymnosperms and 58% of the angiosperms (but only 8% of the monocotyledons that were tested) were susceptible to infection by wild-type *Agrobacterium*. In the past few decades, the number of plant species reported to be transiently or stably transformed by *Agrobacterium* has grown rapidly, mainly owing to a better understanding of *Agrobacterium* biology, manipulation of the *Agrobacterium* genome and new developments in tissue-culture techniques for various plant species. Using diverse *Agrobacterium* strains and isolates, recombinant *Agrobacterium* plasmids and a plethora of inoculation and selection techniques, transgenic plants of many species previously thought to be 'recalcitrant' to *Agrobacterium*-mediated genetic transformation (AMGT) are routinely produced today. Moreover, recent discoveries of the roles of plant factors during the genetic-transformation process [3,4] are expected to further increase the *Agrobacterium* host range, for example, by genetic manipulation of plant species that are still recalcitrant [7]. Reflecting this, the mechanism by which *Agrobacterium* transforms its host (Box 1) has been the subject of numerous reviews (e.g. Refs. [4,8]).

The transfer of T-DNA (transferred DNA) from *A. tumefaciens* to plant genomes, by a type IV secretion system (T4SS), most probably resembles DNA transfer between bacteria during conjugation. Indeed, T4SS was found to be functional during conjugative transfer of Ti and other plasmids between *Agrobacterium* strains [9], between *Agrobacterium* and other bacteria [10,11] and between *Agrobacterium* and plant cells [12]. The functional similarity between T4SSs of *Agrobacterium* and other bacteria, such as intracellular pathogens of mammals *Brucella* spp. and *Legionella pneumophila* [13], suggests that *Agrobacterium* can potentially exchange genetic material with non-plant species. Recently, *Agrobacterium* was found capable, under laboratory conditions, of genetically transforming eukaryotic organisms that do not belong to the plant kingdom (Figure 1), ranging from yeast [14] to filamentous fungi and cultivated mushrooms [15] to cultured human cells [16]. This capability opens the way for the use of *Agrobacterium* in biological research and in the biotechnological improvement of non-plant species. In the following sections, we review the recent trends in AMGT of non-plant eukaryotic hosts, emphasizing not only its potential as a tool for genetic engineering but also as a unique research tool for studies of the fundamental

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Box 1. Mechanism of T-DNA transfer from *Agrobacterium* to the host cell genome

After activation by signals secreted by the host plant or by exogenous application of acetosyringone, a phenolic molecule that is a commercially available potent inducer of *Agrobacterium* [20], a subset of *Agrobacterium* virulence (*vir*) genes located on the bacterial Ti plasmid is expressed, leading to the excision of a single-stranded copy of the T-DNA, from the T-DNA region on the Ti plasmid, by a VirD1–VirD2 heterodimer. The single-stranded T-DNA (T-strand), covalently linked at its 5′ end to VirD2, is then exported to the host cell by a T4SS encoded by the *vir*B operon and the *vir*D4 gene [13]. Independently of the VirD2–T-strand complex (immature T-complex), several Vir proteins, VirE2, VirE3, VirF and VirD5, are also exported by the same VirB and VirD4 channel into the host cell cytoplasm [13]. Once inside the host cell, the VirD2–T-strand conjugate is coated with VirE2 molecules and produces a mature T-complex that is then imported into the host cell nucleus with the assistance of several host and bacterial factors, uncoated by targeted proteolysis and integrated by an as yet undetermined mechanism [3,4,55]. The T-DNA transfer is not sequence specific, which enables its use in biotechnology by replacing the native T-DNA genes with any gene(s) of interest.

biological aspects of the genetic-transformation process, eukaryote–bacterium interactions and basic biology of the host organisms.

First encounters: from yeast to human cells

The first report of AMGT of a non-plant host involved cells of the budding yeast *Saccharomyces cerevisiae* [14], whereby a ura$^-$ yeast strain was transformed to ura$^+$ following introduction of the Ura3-encoding gene by T-DNA transfer and integration. At that time DNA transfer by a conjugative mechanism to *Saccharomyces cerevisiae* was already known to be possible from *Escherichia coli* [17,18], but not from other bacterial species. Although it was later shown that genetic transformation of yeast by *Agrobacterium* can also occur by conjugative plasmid transfer [19], this ground-breaking study [14] strongly suggested a similar mechanism for T-DNA transfer in yeast and plant systems. First, the presence of acetosyringone, a plant-produced inducer of the *vir* genes [20], was essential for the transformation process. Second, the T-DNA was protected against nuclease degradation (which is not the case in direct plasmid transformation), suggesting an active function of the *Agrobacterium* Vir proteins in the process. Third, *Agrobacterium* strains mutated in various *vir* genes known to be essential for AMGT of plant species were non-virulent, or showed much attenuated virulence towards yeast cells [14]. Using specially designed *Agrobacterium* binary vectors, several unique features of AMGT of yeast cells were identified. For example, circularization and autonomous replication of T-DNA molecules in the host cells could be achieved by introduction of the yeast 2-μ replication origin into the T-DNA region. Furthermore, T-DNA integration into the host genome by the homologous recombination mechanism was possible if T-DNA contained specific sequences that share homology with the yeast genome [14]. By contrast, no autonomous replication of T-DNA molecules has ever been observed in plant cells, and the integration of T-DNA molecules in plant cells is mostly, if not solely, mediated by illegitimate recombination.

Some years later, *Aspergillus awamori* became the first filamentous fungus to be genetically transformed by *Agrobacterium* [15]. Similar to the process in plants, acetosyringone was essential for transformation, and the integration of the T-DNA molecules, carrying the hygromycin resistance gene under the control of the fungal promoter, was random. In addition, sequence analysis of the junctions between integrated T-DNA and the host genomic DNA had small truncations at one of the T-DNA ends, as is frequently observed in T-DNA integration in plants [15]. It should be noted, however, that the random T-DNA integration, most probably mediated by non-homologous recombination, observed in these experiments was due to the lack of homology between the T-DNA and the fungal genome sequences. Indeed, later studies showed that integration of T-DNA by homologous recombination is possible in filamentous fungi if the T-DNA shared regions of homology with the host genome [21]. Overall, the number of fungal species capable of being transformed by *Agrobacterium* by either of the two T-DNA integration mechanisms (i.e. by homologous recombination and non-homologous end-joining) has dramatically increased in recent years (Figure 1; [22]).

The ability of *Agrobacterium* to transform non-plant eukaryotes genetically is not limited to fungi, and Kunik *et al.* showed that cultured human cell lines (HeLa, HEK293 and neuronal PC12 cells [16]) were transformed by *Agrobacterium* using neomycin-resistance as the selection marker. In their report, the T-DNA transfer and integration into human cells shared most of the features of plant AMGT. Bacterial attachment to the host cell, an essential step in plant transformation by *Agrobacterium*, was similar in human cells and in plant protoplasts. Moreover, *Agrobacterium* mutants in the *chv*A and *chv*B loci (two genes required for *Agrobacterium* attachment to plant cells) were unable to bind to human cells. The mode of T-DNA integration into the human genome was also essentially similar to integration of DNA into the plant genome (i.e. the integration event occurred at the T-DNA borders), suggesting *bona fide* T-DNA transfer rather than conjugative transfer of the Ti-plasmid [16]. As in yeast, the importance of acetosyringone and the lack of transformation with several *Agrobacterium vir* gene mutants clearly indicated the role of the *Agrobacterium* virulence system in T-DNA transfer from *Agrobacterium* to human cells.

These pioneering experiments demonstrated that the *Agrobacterium* T-DNA transfer is not limited to plant hosts: other eukaryotes, such as yeast and other fungi and human cells, can be transformed by *Agrobacterium*, under laboratory conditions. Moreover, the mechanism of this DNA-transfer process, which relies on the *Agrobacterium* Vir proteins, is essentially the same in plant and non-plant hosts. However, the mechanism by which the T-DNA molecule integrates into the host genome is most likely to be dictated by the nature of the host organism and the nucleotide sequence of the T-DNA. Comparative

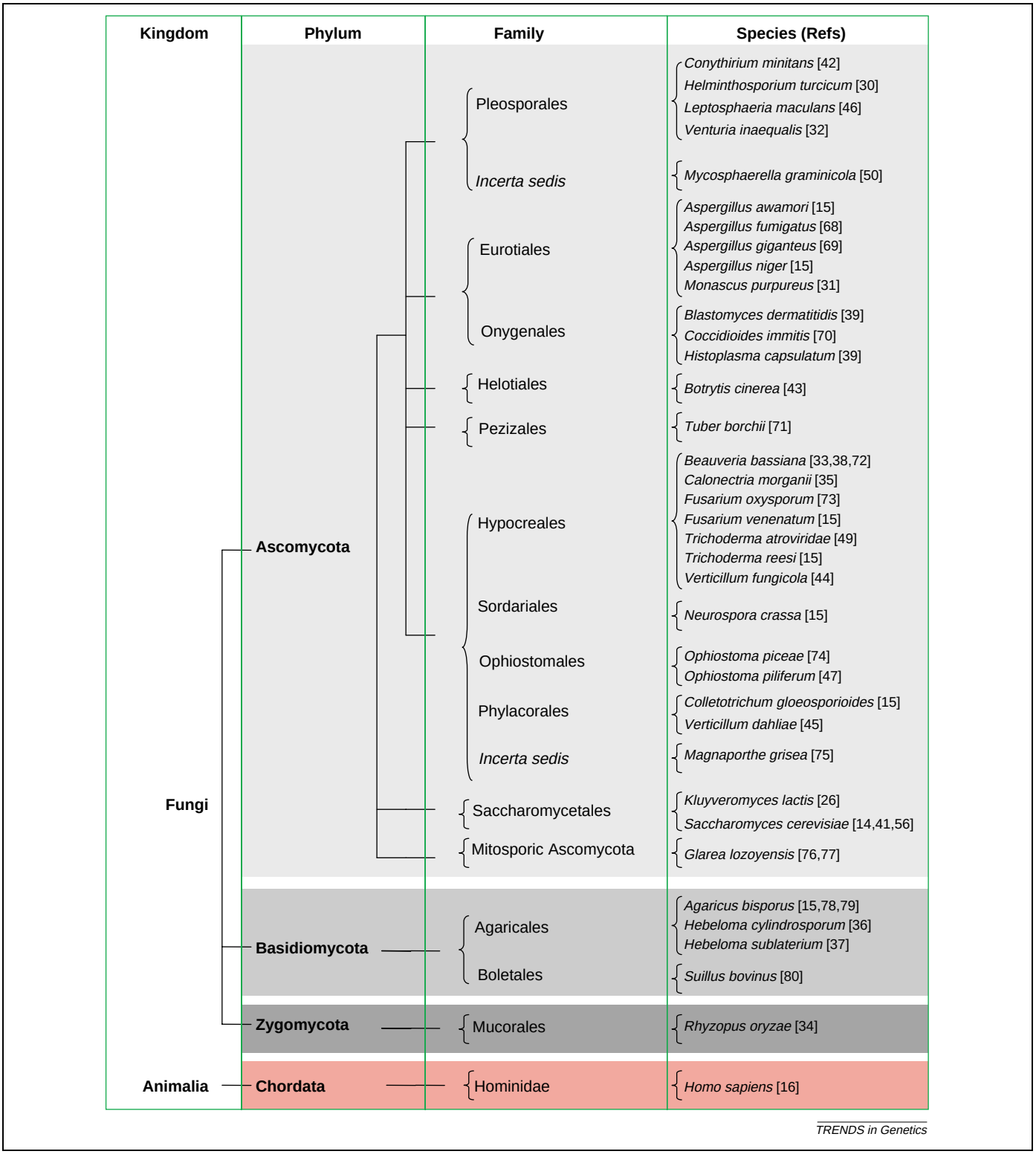

Figure 1. A list of non-plant species that can be genetically transformed by *Agrobacterium*. Under laboratory conditions, *Agrobacterium* can transform human cells and numerous species that belong to the three main phyla of fungi. Most likely, other non-plant eukaryotic cells will found that can function as *Agrobacterium* hosts, opening the way for new applications and scientific advances.

genome-sequence analyses have suggested the importance of horizontal gene transfer early in molecular evolution [23]; the prokaryote-to-eukaryote DNA transfer represented by the AMGT of different eukaryotic hosts could be one of the mechanisms involved in this process. The next challenge for *Agrobacterium* (and for scientists working in genetic engineering) is the transfer of DNA to other types of eukaryotes, such as unicellular protozoa, algae or insects, which should be achievable based on the results obtained with fungal and human cells.

Box 2. Basic features of *Agrobacterium*-mediated genetic-transformation protocols for production of transgenic plant and non-plant eukaryotic organisms

Agrobacterium-mediated genetic transformation of plants follows a relatively rigid set of protocols, established over several decades of transgenic research. These protocols involve choosing suitable regenerative plant tissues and growth media, using appropriate selectable marker genes that are controlled by a promoter expressed in the host organism and screening transgenic plants expressing the gene of interest integrated predominantly by non-homologous recombination (Table I). Although the *Agrobacterium*-mediated genetic transformation of non-plant species follows the same basic steps, transformation protocols of non-plant species are more flexible not only in the choice of the target tissues, cells and selection systems but also in the outcome of the transformation process itself (i.e. the transgene can be integrated by homologous and non-homologous recombination).

Table I. AMGT of plant and non-plant hosts: differences and similarities

Plant hosts	Non-plant hosts
Virulence induction	
Induction of the *Agrobacterium vir* region is the essential step for production and transport of the T-strand and Vir proteins into the host cell	
Wounded tissues supply phenolic inducer molecules, making addition of exogenous acetosyringone optional	*vir* gene inducers (e.g. acetosyringone) must be supplied exogenously
Target tissue	
The choice of the target tissue is usually dictated not only by its susceptibility to *Agrobacterium* but also by its ability to regenerate or develop into a new, fully developed organism	
Somatic, regenerative tissues are the main target tissues, with the exception of several flower-dip transformation protocols where the female reproductive tissues probably serve as the target tissue	Somatic or reproductive cells and organs are used as targets, including protoplasts, intact cells, hyphae, spores, conidia and mycelial tissues
Selection	
Different techniques and selection markers can be employed for discriminating between transformed and non-transformed tissues. They include visual screening and screening for resistance to selection agents, classified into four major types of markers:	
Conditional-positive markers [63]	
The most commonly used markers in plant genetic transformation, which include resistance to antibiotics (e.g. hygromycin and kanamycin), herbicides (e.g. phosphinothricin), toxic metabolite analogs (e.g. 2-deoxyglucose) and non-toxic growth regulators	Resistance to antibiotics (e.g. hygromycin) and herbicides (e.g. phosphinothricin)
Conditional-negative markers [63]	
Result in death of transformed cells, can cause developmental defects, are used to ablate specific cell types or, in combination with conditional-positive markers, to screen against certain genetic events, includes toxic proteins (e.g. cytosine deaminase and dehalogenase)	Not reported
Non-conditional-positive markers [63]	
Visually detectible morphological or developmental alterations (e.g. spontaneous shoot regeneration and 'hairy' roots)	Not reported
Complementation of function	
Host gene complementation strategies are typically not applicable for plants	Rescue of conditional (e.g. auxotrophic and temperature sensitive) mutants
Integration pattern	
The T-DNA integration pattern dictates the potential application of *Agrobacterium*-mediated genetic transformation of host species	
Random T-DNA integration in plants restricts the use of *Agrobacterium* to production of transgenic plants and random T-DNA insertion libraries. No efficient system exists for plant gene targeting or plant gene replacement	The host genotype (i.e. genes involved for homologous or non-homologous recombination) and the presence of T-DNA sequences homologous to the genomic sequences of the host determine the mechanism of T-DNA integration in many non-plant species, enabling the use of *Agrobacterium* not only for production of transgenic organisms and random T-DNA insertion libraries but also for gene targeting

Agrobacterium T-DNA transfer as a tool for genetic engineering of non-plant organisms

The genetic transformation of fungal species is important, not only scientifically but also economically; genetically modified yeast and non-yeast fungi account for most of the industrial production of recombinant proteins [24]. The availability of several reliable and efficient protocols for the genetic transformation of yeast [25] reduces the importance of AMGT as a new transformation method for *S. cerevisiae*; however, AMGT could provide an important alternative approach for hard-to-transform yeast species [26,27] (discussed in the next section). For non-yeast fungi, various transformation protocols have been developed and successfully employed with different species (e.g. biolistics, electroporation, polyethylene glycol (PEG) and $CaCl_2$, and $LiCl_2$ [28]). Nevertheless, the use of AMGT has brought a great deal of improvement to genetic-transformation methods in fungi [22,29]. For example, the first successful genetic transformation of several fungal species that were not transformable by traditional methods could only be achieved with AMGT [30–33], and the use of AMGT with many other fungal species (Figure 1) has several important advantages over most existing protocols.

The major advantage of AMGT over traditional transformation methods is the exceptional versatility of

the physical condition and developmental state of the host cell that can be employed in the transformation experiments [30] (Box 2). Although many of the conventional methods usually require the preparation of protoplasts (a practice that is often a source of problems with respect to the protoplast production, cell-wall regeneration and selection of transformants), AMGT can be used with a wide range of cells and tissues: protoplasts, intact cells, hyphae, spores, conidia and mycelial tissues have all been successfully transformed by *Agrobacterium* [22,29]. A second advantage is that, unlike conventional transformation methods, AMGT produces transformants that are mitotically stable and contain mostly single transgene insertions. In *Rhyzopus oryzae*, for example, only mutants obtained by AMGT showed integration of a single T-DNA copy and remained stable, even after the selection pressure was removed, whereas the PEG and $CaCl_2$ method resulted in mutants carrying multiple vector molecules, which replicated autonomously and were mitotically unstable [34]. Similarly, in *Beauveria bassiana*, 90% of the transformants obtained by AMGT contained a single copy of the integrated T-DNA, whereas transformants produced by the PEG method exhibited mostly multi-local integration [33]. Third, transgenic yields following AMGT are generally greater than those following other transformation methods. For example, when compared directly with the PEG and $CaCl_2$ method (the most widely used technique for transformation of fungi), AMGT was 600 times more efficient in *A. awamori* [15] and 140 times more efficient in *Calonectria morganii* [35]. However, the overall efficiency of AMGT in non-plant systems remains significantly lower than that observed for plant hosts, suggesting that, in nature, AMGT of non-plant species either does not occur or represents an extremely rare event.

Among the different factors influencing the efficiency of AMGT of fungal species, two were clearly identified. First, the presence of acetosyringone was necessary during the *Agrobacterium*-fungus co-cultivation step, albeit not in the bacterial culture before inoculation [15,36]. Second, the overall transformation efficiency was strongly dependent on the ratio of bacterial to fungal cells during co-cultivation (e.g. Ref. [37]). The main selection agent used in fungal AMGT protocols is the antibiotic hygromycin, which is also commonly used to select for transgenic plants. However, other selection markers have also been used. For example, in *B. bassiana* [38], which is resistant to hygromycin, phosphinotricin (or BASTA) – a herbicide also employed in plant genetic transformation – was used for selection [38]. A unique method of selection that is not used in plant species, but is most useful in yeast and fungal transformation, is complementation of gene function. This strategy has the advantage of avoiding the introduction of an antibiotic-resistance gene or foreign DNA (which is an important issue for food-related applications), but requires the availability of fungal strains or species that are deficient in specific nutritional requirement functions. For example, uracil synthase complementation was used with uracil auxotrophic lines of *Blastomyces dermatitidis* and *Histoplasma capsulatum*, and was even found to be more efficient than hygromycin selection in these two species [39].

Agrobacterium-mediated genetic transformation as a genomic tool for non-plant species

In plants, T-DNA integration occurs almost exclusively by illegitimate recombination, which results in random insertion of T-DNA molecules into the genome [40]. In yeast and fungi, however, homologous recombination is the major pathway for DNA integration, provided that the integrating DNA shares sequence homology with the host genome. This feature raises the possibility of choosing between random or targeted integration of the foreign DNA by using sequences that are either dissimilar or similar, respectively, to the host genome sequences, thus providing genomic tools for insertional mutagenesis or specific gene replacement. Indeed, T-DNA molecules could be integrated into yeast cells by illegitimate [41] or homologous recombination [26], depending on their sequence homology with the host genome. Three major features of T-DNA integration by illegitimate recombination make it a more efficient tool for insertional mutagenesis of non-yeast fungi [29] than other transformation methods. First, T-DNA molecules integrate randomly in the host DNA, ultimately saturating the entire genome. Second, mostly single copy (or a few copies) of T-DNA is integrated in each transformant. Third, the transformation efficiency is generally greater than that achieved with other methods. Hence, insertional mutagenesis using T-DNA integration has been undertaken in several fungal species. For example, *Coniothyrium minitans*, a mycoparasite used as a biocontrol agent of plant pathogens, was chosen as a host to compare AMGT with the more classical PEG and $CaCl_2$ transformation methods combined with restriction enzyme-mediated integration (REMI) for the generation of a bank of insertional mutants [42]. AMGT yielded both more efficient transformation and a greater proportion of single insertions (40% for AMGT versus 8% for the PEG-$CaCl_2$-REMI combined method); pathogenicity mutants were identified in the mutagenized population obtained by AMGT. Other reports on *B. dermatitidis*, *H. capsulatum* [39], *Hebeloma cylindrosporum* [36], *Botrytis cinerea* [43] and *B. bassiana* [33] confirmed that AMGT is a promising tool for functional genomics in fungi, where other methods of DNA insertion are generally unsatisfactory.

AMGT was also more efficient than electroporation in achieving targeted DNA integration in the yeast *Kluyveromyces lactis* [26]. It is worth noting that although targeted integration is highly efficient with *S. cerevisiae* or *Schizosaccharomyces pombe* using classic genetic-transformation protocols, it is more difficult with other species of yeast, such as *K. lactis*, where AMGT is rapidly becoming the method of choice. Moreover, T-DNA integration by homologous recombination was efficient in the filamentous fungus *A. awamori* [21], and this feature was used for targeted integration at the *pyr*G locus in the host genome, enabling selection of transgenic fungi without the introduction of a bacterial antibiotic gene or other foreign DNA as selection markers. This technique was later shown to be preferable to PEG and $CaCl_2$, because of better efficiency of targeted integration [22]. Targeted integration mediated by AMGT was also successfully applied to other fungal species, mainly for reverse genetics studies of specific gene functions [44–50]. For example, in

Coccidioides posadasii, a fungal human pathogen, the gene encoding 1,3-β-glucan synthase (*FKS1*, an enzyme implicated in cell-wall biosynthesis) was specifically replaced using AMGT with a construct containing the hygromycin-resistance gene flanked by 5′ and 3′ non-coding sequences of the host *FKS1* gene. Cell lines with disrupted *FKS1* represented 25% of the transformants, were mitotically stable and exhibited a specific phenotype, such as abnormal swelling of hyphal elements, suggesting a role for this gene in maintaining the integrity of the cell wall [51].

The use of non-plant hosts to advance our understanding of the *Agrobacterium* T-DNA-transfer mechanism

The similarities and differences between plant and non-plant hosts, with respect to their interaction with the bacteria during infection, can shed light on different aspects of the mechanism governing *Agrobacterium* infection. Among non-plant hosts, yeast cells represent the most attractive system to study AMGT, because they grow rapidly, are easily manipulated and their transformants are easily isolated by colony formation on selective medium, enabling precise quantification of the transformation efficiency. Moreover, comprehensive collections of yeast mutants are available and can be used for functional studies of AMGT. This approach was used to demonstrate that adenine auxotrophic yeast mutants were hypersensitive to AMGT when grown on a medium deprived of adenine [52]; these observations were then translated to plant hosts in which purine-biosynthesis inhibitors induced hypersensitivity to AMGT [52].

S. cerevisiae mutants were also used to study the genetic requirements for homologous and illegitimate integration of T-DNA [53,54]. T-DNA integration by homologous recombination was affected by Rad51 and Rad52, but not Rad50, Mre11, Xrs2, Lig4 or Ku70, whereas T-DNA integration by illegitimate recombination involved Ku70, Rad50, Mre11, Xrs2, Lig4 and Sir4. Moreover, homologous and illegitimate modes of integration could be uncoupled and selectively inhibited by disruption of specific genes; disrupting the *Ku70* gene blocked integration by the illegitimate recombination pathway [53], whereas in the absence of Rad52 the homologous recombination pathway was blocked [54]. This work helped develop the latest model for T-DNA integration into the host genome [55], and suggested that Ku70 and Rad52 are key enzymes that specify the integration pathway, homologous or non-homologous, by which the T-DNA will be inserted into the host genome.

Several studies also used yeast as model hosts to examine the roles of the bacterial *vir* genes in AMGT and compare them with the functions of plant *vir* genes in AMGT. Specifically, the *vir* genes, involved in sensing plant signals (*vir*A, *vir*G), T-strand synthesis (*vir*D2) and translocation to the plant cell (*vir*B1, *vir*B4, *vir*B7, *vir*D4), are as essential for the transformation of *S. cerevisiae* as they are for transformation of plants [14,56]. Nevertheless, a *vir*E2 *Agrobacterium* mutant still retained ~10% of its transformation efficiency with yeast [14], although this gene was essential for AMGT of several plant species (Ref. [4] and references therein).

Similar experiments, performed with the filamentous fungus *A. awamori*, showed that mutations in *vir*A, *vir*G, *vir*D2, *vir*D4, *vir*D1, *vir*D2 and in most genes of the *vir*B locus completely disrupted T-DNA transfer, and mutations in *vir*E2, *vir*C2 and *vir*B1 exhibited 50%, < 10% and ~5% AMGT efficiency compared with the control, wild-type *Agrobacterium* strain, whereas mutations in *vir*E3, *vir*F and *vir*H had virtually no effect on transformation efficiency [57]. Moreover, partial mutants in the *vir*D2, *vir*C2 and *vir*E2 genes showed an unusual T-DNA integration pattern, with more deletions in the integrated T-DNA, in addition to strongly reduced transformation efficiency [57]. The requirement of most essential virulence operons and genes, including the *vir*E operon and the chromosomal genes *chv*A and *chv*B, was demonstrated for AMGT of human cells [16], thus indicating the possible similarities, and differences, between AMGT of plant, fungal and human cells.

Box 3. The risks and promises of studies of *Agrobacterium*–host interactions

The risks

Will *Agrobacterium* transfer genes to non-plant species outside the laboratory?

The ever-expanding range of known hosts for *Agrobacterium,* which includes many plant and non-plant species, raises the concern of accidental release into the environment of genetically modified *Agrobacterium* species and subsequent gene transfer to soil- and plant-borne fungi and other non-plant organisms. The extremely low efficiency of AMGT of the non-plant species even under the optimized laboratory conditions suggests that this process might not occur in nature. Nevertheless, the issue of cross-species gene transfer should be further studied; caution and appropriate biocontainment should be exercised in the use of *Agrobacterium* for genetic engineering of plants and non-plant species.

Furthermore, *Agrobacterium* species are now recognized as rare and opportunistic human pathogens affecting mostly immunocompromised patients [64,65]. Isolates from patients affected by bacteremia, peritonitis and endocarditis enabled the identification of *Agrobacterium radiobacter* (an 'avirulent' *Agrobacterium* species) as the main disease-causing agent (e.g. Ref. [64]). In addition, *Agrobacterium tumefaciens* and *Agrobacterium vitis* were isolated from hospitalized patients [65], although the potential link between the *Agrobacterium* virulence system and the disease symptoms in humans has not been studied.

The promises

Will 'gene-shuffling' between species become a new approach to optimize the use of *Agrobacterium* as a tool for genetic engineering of plant and non-plants organisms?

AMGT of most economically-important plant and non-plant species is an extremely inefficient process: only few of the host cells are initially infected, and T-DNA integration and stable gene expression occur in an even smaller fraction of the infected cells. Genetic modification of the host by introducing genes from other organisms that facilitate AMGT into its genome is an extremely promising approach for improving transformation of plant species that are normally recalcitrant to AMGT [7]. For example, the expression of genes involved in AMGT of plants [66] in non-plant species can be used for improving the genetic transformation of the non-plant organisms. However, genes of non-plant species might be used to alter the AMGT outcome in plant hosts. Specifically, transgenic plants that express yeast genes involved in homologous recombination enable high-frequency sequence-specific integration of T-DNA, which is normally an extremely rare event in plants [67].

Non-plant species are also useful for functional assays to dissect specific steps of the AMGT process. For example, a Cre-recombinase reporter assay for translocation (CRAFT) of proteins from *Agrobacterium* to host cells was originally developed for *Arabidopsis* [58] and then adapted for *S. cerevisiae* [59]. In this assay, a fusion of Cre recombinase with the tested protein is expressed in *Agrobacterium*, and the export of the fusion product is monitored by Cre-activated expression of a selectable gene in the host cell. This approach was used to reveal the export of VirE3 from *Agrobacterium* to yeast cells [59], which was later demonstrated in plant cells [60,61].

Future directions

AMGT provides significant advantages over other available genetic-transformation protocols in many non-plant species [22]. Specifically, its efficiency is greater than that of other genetic-transformation methods in most fungal species, and it generally results in single and stable integration events. Other eukaryotic organisms are probably susceptible to genetic transformation by *Agrobacterium*, and the demonstration of this capability would support the idea that DNA transfer between different kingdoms, specifically from bacteria to eukaryotes, has represented an important process throughout biological evolution. Moreover, T-DNA is transferred through a T4SS channel, which is widespread among bacteria, suggesting that DNA transfer to eukaryotic hosts might not have been restricted to *Agrobacterium*.

Non-plant species have also provided us with new tools and methods for studying the *Agrobacterium*–host interaction and the AMGT process. The development of additional non-plant model systems and tools will help improve and control the transformation process in plants and extend the host range of *Agrobacterium* to other species (Box 3). The capability of *Agrobacterium* to export proteins to its host cells independently of the T-DNA transfer [58,62] can be exploited as a new biotechnological tool for genetic engineering of both plant and non-plant organisms.

Concluding remarks

The studies discussed in this article ensure a bright future for *Agrobacterium* as a universal tool for genetic transformation of all organisms. One can suppose that this insatiable bacterium, always eager to share pieces of DNA with new 'friends', will continue jumping from one organism to the next for a short 'one-night stand' or adding new partners to the old ones for a long-lasting polygamous relationship. Au suivant!

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