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Agrobacterium tumefaciens-mediated transformation of yeast

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ABSTRACT ***Agrobacterium tumefaciens*** **transfers a piece of its Ti plasmid DNA (transferred DNA or T-DNA) into plant cells during crown gall tumorigenesis.** ***A. tumefaciens*** **can transfer its T-DNA to a wide variety of hosts, including both dicotyledonous and monocotyledonous plants. We show that the host range of** ***A. tumefaciens*** **can be extended to include** ***Saccharomyces cerevisiae.*** **Additionally, we demonstrate that while T-DNA transfer into** ***S. cerevisiae*** **is very similar to T-DNA transfer into plants, the requirements are not entirely conserved. The Ti plasmid-encoded** ***vir*** **genes of** ***A. tumefaciens*** **that are required for T-DNA transfer into plants are also required for T-DNA transfer into** ***S. cerevisiae,*** **as is** ***vir*** **gene induction. However, mutations in the chromosomal virulence genes of** ***A. tumefaciens*** **involved in attachment to plant cells have no effect on the efficiency of T-DNA transfer into** ***S. cerevisiae.*** **We also demonstrate that transformation efficiency is improved 500-fold by the addition of yeast telomeric sequences within the T-DNA sequence.**

Agrobacterium tumefaciens causes crown gall tumors in plants by transferring a segment of DNA (transferred DNA or T-DNA) from its tumor-inducing (Ti) plasmid to the nucleus of plant cells. The T-DNA becomes integrated into the plant nuclear genome where it functions to give rise to the characteristic tumor (reviewed in refs. 1 and 2). This process depends on the induction of a set of Ti plasmid-encoded virulence (*vir*) genes. *vir* genes are induced via the *vir*A/*vir*G two-component regulatory system which senses monosaccharides and phenolic compounds from wounded plants (reviewed in ref. 3). The T-DNA is a single-stranded DNA molecule produced by a *virD1/D2*-encoded site-specific endonuclease that nicks within two 24-bp direct repeat sequences on the Ti plasmid (4). These repeats, termed border sequences, flank the T-DNA. Following cleavage and excision, the T-DNA is coated by the single-stranded DNA binding protein VirE2 (5), and the resulting T-DNA complex is transferred to the plant cell.

The mechanism by which the T-DNA complex is transported through the inner and outer bacterial membranes and into the plant cell is not well understood. It is believed on the basis of several lines of evidence that the VirB proteins and VirD4 are involved in T-DNA transport (reviewed in ref. 6). Once the T-DNA complex enters the plant cell, it is targeted to the nucleus via nuclear localization sequences in the VirD2 and VirE2 proteins (7, 8). Upon entering the nucleus, the T-DNA is integrated into the plant genome by illegitimate recombination, a process likely mediated by host factors (9).

The study of host factors involved in T-DNA transfer has been difficult and would be greatly facilitated by the availability of a host model amenable to genetic manipulation. Given the similarities between T-DNA transfer and conjugative transfer of broad-host-range plasmids (reviewed in refs. 1, 6, and 10), we set out to determine if *A. tumefaciens* can transfer T-DNA to the yeast *Saccharomyces cerevisiae.* It has been demonstrated that *Escherichia coli* can conjugate with both *S. cerevisiae* (11, 12) and *Saccharomyces kluyveri* (13). Bundock *et al.* (14) recently demonstrated T-DNA transfer from *A. tumefaciens* to *S. cerevisiae.* In this report, we confirm many of their observations and show additionally that *A. tumefaciens*-mediated transformation of yeast requires most but not all of the same conditions that are required for T-DNA transfer into plant cells. We also demonstrate that the efficiency of transformation is greatly influenced by the presence of stabilizing factors on the T-DNA.

MATERIALS AND METHODS

Strains. Bacterial and yeast strains used in this study are listed in Table 1. *E. coli* cells were grown on LB medium (21) supplemented with appropriate antibiotics to maintain plasmids: 100 μg of carbenicillin or 25 μg of kanamycin per ml. Cultures of *A. tumefaciens* were grown in mannitol glutamate Luria salts medium (MG/L) or induction broth as described (22), except induction broth ($IBPO_4$) was buffered to pH 5.5 with 62.5 mM potassium phosphate. Antibiotics (carbenicillin and/or kanamycin) were added to final concentrations of 100 μg/ml.

S. cerevisiae was maintained on YPD medium (23). Recipients from the transformation assay were recovered on minimal (or B) medium (23). B medium was supplemented with uracil (20 μg/ml), lysine (30 μg/ml), tryptophan (40 μg/ml), and adenine (40 μg/ml) (23) to ensure growth of the yeast cells. Transformants were selected on the same medium lacking tryptophan. To prevent growth of the donor *A. tumefaciens* cells on B medium plates, 200 μg of cefotaxime per ml was included. The cocultivation medium (CM) consisted of $IBPO_4$ (pH 5.5) containing lysine, tryptophan, adenine, and uracil at the concentrations given above for B medium, as well as 100 μg each of carbenicillin and kanamycin per ml.

Genetic Techniques. All recombinant DNA techniques were performed as described by Ausubel *et al.* (23) or Sambrook *et al.* (21). Enzymes were purchased from BRL, New England Biolabs, or Boehringer Mannheim. Competent *E. coli* DH5α cells (21) were purchased from BRL and transformed according to the manufacturer's protocol. DNA was introduced into *A. tumefaciens* by electroporation as described (22).

Plasmid Constructions. The salient features of pKP506, pKP509, and pKP510 are diagrammed in Fig. 1. pSc4128, a gift from B. Brewer (University of Washington) (24) consists of a 7.7 kb *Xho* I–*Sal* I yeast DNA fragment containing *ARS1* and the *TRP1* and *GAL3* genes ligated into the *Sal* I restriction site of pBR322. A 2.25-kb *Sph* I fragment containing a kanamycin resistance gene (*neo*) and two yeast telomeric sequences in an inverted orientation was isolated from $pYLPV_K$, a derivative of pYLPV (25) in which the *leu2-d* gene has been replaced by the *neo* gene (V. Zakian, personal communication). The 2.25-kb fragment was treated with the Klenow fragment of

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Abbreviations: AS, acetosyringone; T-DNA, transferred DNA; CHEF, clamped homogeneous electric fields.

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Table 1. Strains

Strain	Description	Reference
A. tumefaciens		
EHA105	C58 strain containing pTiBo542 harboring a T-DNA deletion	15
A348	C58 strain containing pTiA6Nc	16
At10002	A348 Δ*virD1,D2* Ω*nptII*	This study
At12506	A348 *virD4*::Tn3HoHo1, MX367 cured of pPH1JI	17, 18
At11067	A348 *virB1*::Tn3HoHo1, MX243 cured of pPH1JI	17, 19
S. cerevisiae		
YPH45	*MAT***a** *ura3-52 lys2-801*^{amber} *ade2-101*^{ochre} *trp1-Δ1*	20
Y104	*MAT***a** *ura3-52 lys2-801*^{amber} *ade2-101*^{ochre}	20

DNA polymerase I to fill in the ends. This blunt-ended fragment was ligated into the *Nru* I site of pSc4128 to create pKP505. The *neo* gene was excised from pKP505 by using *Kpn* I, and the large fragment containing the *TRP1* gene and *ARS1* flanked by yeast telomeres was inserted between the T-DNA borders of the *A. tumefaciens* binary vector pBIN19 (26), previously cleaved with *Kpn* I, thereby creating pKP506.

To construct a binary control vector that lacked the T-DNA border sequences, pBIN19 was cleaved with *Bgl* II, and the large fragment was isolated. The vector p34E (27), which contains a duplicate of the pUC multiple cloning site in an inverted orientation, was digested with *Bam*HI and *Bgl* I (to reduce the frequency of p34E religation) and ligated to the large fragment isolated from the *Bgl* II-digested pBIN19 to form pKP507. The large *Kpn* I fragment from pKP505 was then ligated into *Kpn* I-cleaved pKP507, resulting in the T-DNA-borderless plasmid pKP509.

The plasmid containing *ARS1* and the *TRP1* gene, without telomeres, between T-DNA borders (pKP510) was constructed by linearizing pSc4128 with *Nru* I and inserting the entire plasmid into pBIN19 that had been cleaved with *Sma* I.

Construction of *A. tumefaciens virD1/D2* Deletion Mutant. The *Hin*dIII–*Sph* I fragment of pTiA6 containing *virD3* and a portion of *virD4* (28) was ligated into the *Hin*dIII and *Sph* I sites of pEMBL130(+) (29), to generate pKS121. The *Sma* I–*Pst* I fragment containing the *virD/virC* promoter and a portion of *virC* (4, 28) was ligated into the *Hin*cII and *Pst* I sites of pEMBL130(+), to generate pKS122. The *Hin*dIII–*Xho* I fragment from pKS122 was ligated to the *Hin*dIII and *Sal* I sites of pKS121, to generate pKS123. pKS123 now contains the *virD/virC* promoter and *virD3* flanking a unique *Hin*dIII restriction site. The *nptII* gene with its promoter was excised from Tn*5* by using *Sal* I and *Hin*dIII. The *Sal* I site was converted to a *Hin*dIII site with appropriate linkers (18), and the fragment was ligated into the *Hin*dIII site of pKS123, to generate pKS124. This plasmid contains no coding sequence for VirD1 and is missing the coding sequence for the first 391 amino acids of VirD2 (33 remaining). The *virD1/virD2* deletion and *nptII* insertion were placed into the Ti plasmid via marker exchange (22) by electroporating strain A348 with pKS124, selecting for kanamycin resistance, and screening for carbenicillin sensitivity. The resulting strain is At10002.

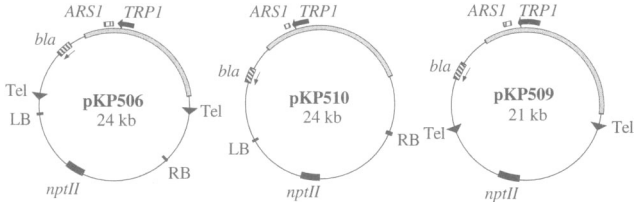

FIG. 1. Plasmids used in this study. For complete construction details see *Materials and Methods.* Abbreviations: *ARS1*, yeast autonomous replicating sequence 1; *TRP1*, yeast tryptophan gene; Tel, yeast telomeric sequences; LB and RB, *A. tumefaciens* left and right T-DNA borders, respectively; *nptII*, kanamycin-resistance gene; *bla*, carbenicillin-resistance gene.

Transformation Protocol. Yeast cells were prepared for transformation by inoculating a single colony from a fresh YPD plate into 5 ml of YPD broth. The cells were grown overnight at 30°C with shaking. The cells were then diluted 20-fold with fresh YPD medium and grown for an additional 6 h. The cells were harvested by centrifugation, washed once with $IBPO_4$ [no acetosyringone (AS)], and resuspended at a final cell concentration of 1×10^9 cells/ml. *A. tumefaciens* cells were grown overnight in MG/L medium containing the appropriate antibiotics. The cells were pelleted, resuspended in an equal volume of $IBPO_4$ (with or without 100 μM AS), and incubated for an additional 6 h. The cells were harvested and resuspended in the same medium to a concentration of 1×10^{11} cells/ml. The cocultivation of bacteria and yeast was initiated by mixing 50 μl each of the donor and recipient cells at a ratio of 100 *A. tumefaciens* cells to 1 yeast cell. The cell mixture was deposited by using vacuum filtration on a 25-mm diameter nitrocellulose filter (0.45-μm pore diameter) (Schleicher & Schuell) which was then placed on CM plates with or without AS and incubated for 72 h at 22°C. The cells were washed off the filters with 1 ml of TNB (20 mM Tris·HCl, pH 7.5/10 mM NaCl), and 100 μl of the appropriate dilutions was plated on B medium plates with or without tryptophan to recover recipients or select for transformants, respectively. The plates were incubated at 30°C, and the colonies were counted after 3–4 days.

Yeast DNA Isolation and Clamped Homogeneous Electric Fields (CHEF) Gel Analysis. Yeast total DNA was isolated essentially as described (23). DNA was analyzed by using a Bio-Rad CHEF DRIII variable angle pulsed field electrophoresis system. Solution containing ≈25 μg of yeast DNA was mixed with an equal volume of 2% pulsed-field certified agarose in 0.5× TBE (1× TBE = 89 mM Tris buffer/89 mM boric acid/2 mM EDTA) and placed in the wells of a 1% agarose gel. Electrophoresis conditions consisted of an initial switch time of 0.1 s, a final switch time of 2.5 s, 6 V/cm, 44°C, 0.5× TBE, and an included angle of 120° for 12.3 h. The DNA was transferred for 2 h to a Nytran Plus positively charged nylon membrane (Schleicher & Schuell) by using a VacuGene transfer apparatus (Pharmacia) and 1.5 M NaCl/0.5 N NaOH as the transfer buffer. The membrane was probed with an internal 650-bp *Xba* I–*Pst* I fragment of the *TRP1* gene on plasmid pKP506. The probe was labeled, and the blot detected with the ECL random prime labeling and detection system (Amersham) by following the manufacturer's protocols.

RESULTS

***A. tumefaciens* Transfers T-DNA into Yeast.** Initially, we set out to determine whether *A. tumefaciens* could transfer T-DNA into yeast cells and if this transfer had the same requirements as transfer into plant cells. Since the T-DNA complex is linear (30), we first established a system for DNA transfer in which the linear DNA could be stably maintained in the recipient yeast cell, thereby removing any requirement for integration into the chromosome. pKP506 (Fig. 1) is a pBIN19-based vector containing the yeast *TRP1* gene and an associated origin of replication, *ARS1*, between the T-DNA borders. The *TRP1* gene allowed for selection of tryptophan prototrophs from recipient Trp^- auxotrophs, while *ARS1* allowed the transferred linear DNA to replicate extrachromosomally. Since telomeres are required for maintenance of linear DNA molecules (31), yeast telomeric sequences were engineered onto the ends of the yeast DNA within the T-DNA borders (Fig. 1). Under conditions that induce the *A. tumefa-*

ciens virulence genes, T-DNA transferred to the recipient cell could be established as a linear, stable, extrachromosomal element due to the *ARS1* locus and the telomeric sequences.

The transformation/cocultivation conditions consisted of donor *A. tumefaciens* cells with wild-type *vir* genes in an induction medium composed of monosaccharides, low pH, and the *vir*-inducing compound AS. When yeast were cocultivated with strain EHA105 (pKP506) under the transformation conditions described above, a transformation frequency of 3×10^{-3} was observed (Table 2). No transformants were detected when recipient cells were incubated in cocultivation conditions in the absence of any donor cells or when EHA105 without a binary vector was used as the donor strain. No spontaneous Trp^+ revertants were observed because the Trp^- phenotype results from a deletion of the entire *TRP1* gene. Similarly, no transformants appeared when 5 μg of the plasmid pKP506 was added directly to the recipient cells, suggesting that neither the cocultivation conditions nor the presence of EHA105 makes the yeast cells competent to take up DNA directly. EHA105 (pBIN19) also failed to yield Trp^+ recipients. To ensure that transformation of yeast by EHA105 (pKP506) is not due to the uptake of T-DNA strands released from dead *A. tumefaciens* cells, DNase was added to the cocultivation mixture. DNase did not significantly reduce the transformation frequency, leading to the conclusion that the T-DNA is not taken up as free DNA by yeast.

A similar set of experiments using *A. tumefaciens* strain A348 (C58 chromosome, A6 Ti plasmid) as the donor cell demonstrated that yeast transformation is not limited to a specific genetic background of the donor cells. However, the transformation efficiency of the two donor strains differed significantly. The transformation frequency when using A348 (pKP506) as the donor strain was lower by a factor of ≈100 than when EHA105 (pKP506) was the donor (Table 2). The increased transformation frequency obtained when using EHA105 as the donor correlates with previous results which show that A281, the parent of EHA105, is more virulent in dicot transformation than A348 (32).

Requirements for *A. tumefaciens*-Mediated Transformation of Yeast. vir *gene induction.* T-DNA transfer to plant cells is dependent on the presence of specific plant wound signal molecules, such as phenolics and monosaccharides, to serve as inducers of the *vir* genes. To determine if a phenolic inducer is required for *A. tumefaciens*-mediated yeast transformation, the phenolic inducer AS was omitted from both the induction medium in which the *A. tumefaciens* cells were grown and the cocultivation medium. No transformants were observed when the AS was omitted, indicating that *vir* gene induction is necessary for yeast transformation.

T-DNA borders. T-DNA border sequences are necessary for the transfer of T-DNA from *A. tumefaciens* to plant cells but are not required for the conjugative transfer of the Ti plasmid between *A. tumefaciens* strains (S. Farrand, personal communication). To determine whether the *A. tumefaciens*-mediated transformation of yeast depends on T-DNA border sequences, a binary vector lacking T-DNA borders was constructed (Fig. 1). The cocultivation of EHA105 harboring the T-DNA-borderless plasmid pKP509 with YPH45 yielded no transformants, even in the presence of AS (Table 3), indicating that T-DNA border sequences are required for transfer. This finding suggests that transformation by *A. tumefaciens* strains requires a T-DNA complex and that the borders are necessary for processing that T-DNA complex in *A. tumefaciens*.

vir *genes.* Since *vir* gene inducers as well as border sequences are required for transformation, it appears that T-DNA processing is required. To verify this assumption, we used donor strains of *A. tumefaciens* with various *vir* gene mutations to determine if key Vir proteins are required for transformation of yeast.

A *virD1/D2* deletion mutant of A348 (At10002) was constructed to determine if T-DNA processing is required for transformation. Western blot analysis confirmed that this mutation was not polar on other *virD* genes. In addition, the restoration of At10002 virulence by complementation with cloned *virD1/D2* genes demonstrated that the avirulence is the result of the deletion (K.M.S., K. Fullner, and W. Deng, unpublished data). When At10002 (pKP506) was cocultivated with YPD45, no transformants were recovered (Table 3), indicating that T-DNA strand formation is required for transformation. This conclusion is consistent with the observation that T-DNA borders are necessary for yeast transformation (Table 3).

Vir mutants that are deficient in the synthesis of the putative T-DNA transfer channel were also tested as T-DNA donors. *A. tumefaciens* strains At12506(*virD4*$^-$) (33) and At11067(*virB1*$^-$; polar on the complete *virB* operon) (33) containing pKP506 were unable to transform yeast (Table 3). Taken together, these data suggest that T-DNA must be processed by cleavage at the border sequences, and, once formed, the T-strands are

Table 2. *A. tumefaciens*-mediated yeast transformation

Donor strain	Cocultivation additions	Colonies on minimal medium without Trp, cells/ml	Colonies on minimal medium, $\times 10^{-6}$ cells/ml	Transformants per recovered recipient, cells/ml
None	None	0	130	$<7.7 \times 10^{-9}$
None	pKP506, 5 μg DNA	0	142	$<7.0 \times 10^{-9}$
EHA105	None	0	195	$<5.1 \times 10^{-9}$
EHA105	pKP506, 5 μg DNA	0	105	$<9.5 \times 10^{-9}$
EHA105(pBIN19)	None	0	125	$<8.0 \times 10^{-9}$
EHA105(pKP506)	None	440,000*	119	3.7×10^{-3}
EHA105(pKP506)	DNase, 100 ng/ml	180,000*	102	1.8×10^{-3}
A348	None	0	110	$<9.1 \times 10^{-9}$
A348	pKP506, 5 μg DNA	0	120	$<8.3 \times 10^{-9}$
A348(pKP506)	None	7,500†	108	6.9×10^{-5}
A348(pKP506)	DNase, 100 ng/ml	4,000†	84	4.8×10^{-5}

All results are from representative experiments that were repeated a minimum of three times. The recipient strain in all experiments, including those with no donor strain, was *S. cerevisiae* YPH45.
*Colonies were counted from a 10^{-3} dilution of the original culture.
†Colonies were counted from a 10^{-1} dilution of the original culture.

Table 3. Genetic requirements of *A. tumefaciens*-mediated yeast transformation

Donor strain	Relevant donor genotype	AS*	Plasmid features†	Colonies on minimal medium without Trp, cells/ml	Colonies on minimal medium, $\times 10^{-6}$ cells/ml	Transformants per recovered recipient, cells/ml
EHA105(pKP506)	Wild type	+	B, T	440,000‡	119	3.7×10^{-3}
EHA105(pKP506)	Wild type	−	B, T	0	141	$<7.1 \times 10^{-9}$
EHA105(pKP509)	Wild type	+	T	0	126	$<7.9 \times 10^{-9}$
EHA105(pKP509)	Wild type	−	T	0	133	$<7.5 \times 10^{-9}$
EHA105(pKP510)	Wild type	+	B	4,100§	530	7.7×10^{-6}
A348(pKP506)	Wild type	+	B, T	7,500§	108	6.9×10^{-5}
At10002(pKP506)	Δ*virD1D2, virD4*$^+$	+	B, T	0	133	$<7.5 \times 10^{-9}$
At12506(pKP506)	*virD4*$^-$	+	B, T	0	172	$<5.8 \times 10^{-9}$
At11067(pKP506)	*virB1*$^-$	+	B, T	0	153	$<6.5 \times 10^{-9}$

The recipient strain in all experiments was *S. cerevisiae* YPH45.
*+, 100 μM AS included in the cocultivation medium; −, no AS present.
†B, T-DNA border sequences present; T, yeast telomeric sequences present.
‡Colonies were counted from a 10^{-3} dilution of the original culture.
§Colonies were counted from a 10^{-1} dilution of the original culture.

transported through the same apparatus used for the transfer of T-DNA into plant cells.

Chromosomal virulence genes. A. tumefaciens cells with mutations in the chromosomal genes *chvA, chvB*, or *exoC* are defective in either the synthesis or transport of β 1,2-glucan which appears to be required for efficient attachment to and transformation of plant cells (34–40). To determine if the same attachment apparatus is utilized in the transformation of yeast as in the transformation of plants, *chvA*$^-$, *chvB*$^-$, and *exoC*$^-$ mutant strains of *A. tumefaciens* were used as donors in the transformation of yeast strain YPD45. No reduction in yeast transformation efficiency was observed (X.L., unpublished data). This result indicates that, at least in the attachment step, the yeast transformation system does not emulate plant cell transformation.

State of the Transferred T-DNA. The system employed in this study does not require that the transferred T-DNA be integrated into the yeast genome to obtain a stable transformant. The *ARS1* locus and telomeric sequences within the T-DNA border sequences should ensure stable maintenance of the transferred, linear DNA. To determine if the telomeric sequences function as predicted, a binary vector lacking the telomeres (pKP510) was constructed and tested in the transformation assay. When EHA105 (pKP510) was cocultivated with yeast, transformants were obtained at a frequency of 7.7×10^{-6} (Table 3), lower by a factor of 500 than when using the binary vector with the telomeres (pKP506). This result suggests that while integration of the transferred DNA can occur, it does not occur each time there is a transfer event.

To characterize the state of the transferred DNA in the yeast transformants, we first measured the stability of Trp$^+$ prototrophy. If Trp$^+$ prototrophy is conferred by an extrachromosomal, autonomously replicating element, then segregation of colonies to Trp$^-$ auxotrophy should occur following growth in nonselective conditions—i.e., rich media. Conversely, if the T-DNA has integrated into the genome of the transformant, no reversion to Trp$^-$ auxotrophy should be observed. We tested the Trp$^+$ stability of transformants derived by cocultivation with EHA105 (pKP506) and with EHA105 (pKP510). The yeast transformants resulting from the donor EHA105 (pKP506) each demonstrated Trp$^+$ instability (J.D.H., unpublished data). Conversely, all but one of the transformants resulting from the donor strain EHA105 (pKP510) exhibited 100% stability of the Trp$^+$ phenotype. The one transformant resulting from the donor strain EHA105 (pKP510) that was unstable demonstrated a 100% loss of Trp$^+$ prototrophy, indicating a high level of instability of the marker.

These results suggest that the *TRP1* marker is harbored on an extrachromosomal, autonomously replicating element in the transformants resulting from cocultivation with the telomere-containing donor EHA105 (pKP506). The majority of the transformants resulting from cocultivation with the telomere-lacking donor EHA105 (pKP510) exhibited Trp$^+$ stability, consistent with integration of the marker into the yeast genome. The single EHA105 (pKP510)-derived transformant exhibiting Trp$^+$ instability likely harbors the *TRP1* gene on an extrachromosomal element resulting from circularization and ligation of the incoming T-DNA molecule.

To further investigate the state of the T-DNA in the yeast transformants, we isolated total DNA from the above transformants. In addition, DNA was isolated from YPH45 (*trp1*Δ) and Y104 (*TRP1*). Southern analysis of *Eco*RI-cleaved DNA from the Trp$^+$ transformants confirmed that each possessed the expected 1.45-kb fragment containing the *TRP1* gene, while none of them contained sequences that hybridize to the 8-kb *Bgl* II fragment from pBIN19 (K.L.P., unpublished data). This result suggests that only the sequences between the T-DNA borders on pKP506 and pKP510 were transferred to the recipient cells and, therefore, the transformants did not arise from conjugative transfer of the entire plasmid.

Uncleaved total DNA isolated from the transformants, as well as from YPH45 (*trp1*Δ) and Y104 (*TRP1*), was subjected to CHEF gel electrophoresis and transferred to a membrane for Southern analysis. If the DNA is maintained as a linear plasmid with telomeres at the ends, a discrete 13-kb DNA fragment is expected when the membrane is probed with an internal fragment of the *TRP1* gene. However, if the *TRP1* gene is integrated into the chromosome, no distinct DNA bands would be expected to hybridize due to the heterogeneity of DNA fragments produced by shearing during DNA isolation.

In the transformants resulting from cocultivation with EHA105 (pKP506), a distinct DNA band of approximately 13 kb was detected (Fig. 2, lanes 10–15). These bands correspond in size to the large *Kpn* I restriction fragment from pKP506 (Fig. 2, lane 16) which represents the DNA fragment that would be formed upon transfer into yeast. Thus, the transformants derived from EHA105 (pKP506) appear to contain the *TRP1* gene in the form of a linear, autonomously replicating extrachromosomal element.

A distinct *TRP1*-hybridizing fragment was not detected in either Y104, known to have a chromosomal copy of *TRP1* (Fig. 2, lane 1) or any of the transformants produced by using the binary vector lacking telomeric sequences (Fig. 2, lanes 3–9). Taken with the Trp$^+$ phenotype segregation data and the evidence that the *TRP1* sequence is indeed present (see above), this result implies that the *TRP1* gene in most of these transformants has been integrated into the chromosome. One

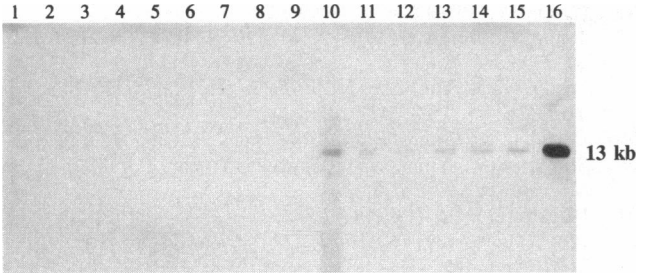

FIG. 2. CHEF gel analysis of transformants. Total, uncleaved yeast DNA was isolated and subjected to CHEF gel electrophoresis. After the DNA was transferred to a nylon membrane, it was probed with an internal fragment of the yeast *TRP1* gene. Lane 1, Y104; lane 2, YPH45; lanes 3–9, transformants derived by using EHA105(pKP510) as the donor; lanes 10–15, transformants derived by using EHA105(pKP506) as the donor; and lane 16, pKP506 cleaved with *Kpn* I.

exception is the transformant that demonstrated 100% instability of the *TRP1* marker, implying its presence on an extrachromosomal element. The absence of any observed bands in the DNA from this transformant might be explained by the fact that circular molecules migrate anomolously in pulsed-field gels and also by the fact that the *TRP1* gene, due to its instability in this transformant, may not be present at a detectable level.

DISCUSSION

The exchange of genetic information between unrelated organisms has been well documented (41, 42). The case of T-DNA transfer from *A. tumefaciens* to plants represents a singular example of genetic transfer in nature since it involves species from different kingdoms. Although our understanding of the mechanism of T-DNA transfer has increased dramatically in the last decade, further advances in this unusual host–parasite interaction will require a concerted effort to unravel the roles that host factors play. The identification of such host factors has been elusive, primarily because of the innate difficulties associated with plant genetics and biology. To circumvent this problem, we set out to establish an *A. tumefaciens*-mediated transformation system involving a host that is more amenable to genetic and biochemical investigation.

Given that different *Saccharomyces* species have been successfully used as conjugative recipients of plasmids from *E. coli* (11–13), we chose *S. cerevisiae* as the host organism for this study. *S. cerevisiae* provides many advantages that can be utilized in such a study. First, it is a well-characterized eukaryotic organism, and its cell biology has been intensively investigated. Second, molecular biological methods and cloning vectors are available, making yeast amenable to genetic manipulation. Third, characterized yeast mutants are available which can be used to analyze eukaryotic factors or structures that may be involved in T-DNA transfer. These advantages make yeast an attractive choice for dissecting the host-factor requirements in T-DNA transfer.

In establishing the transformation assay, the binary vectors were constructed such that the transferred T-DNA would be stably maintained once in the recipient cell. The key advantage to the system described here is that, although homologous recombination between the incoming DNA and the yeast chromosome can take place, the production of transformants does not depend on it. Thus, each yeast cell that receives the DNA may become a Trp^+ prototroph and will not require a recombination event to establish a stable transformant. Consequently, T-DNA transfer, not recombination or integration, is the limiting step in this system.

In a recently published article Bundock *et al.* (14) report *A. tumefaciens*-mediated transformation of *S. cerevisiae* at a frequency of approximately 10^{-6} transformants per recovered recipient and show a requirement for many of the same *A. tumefaciens* virulence genes that are required for plant transformation. In this report, we confirm these observations and describe a yeast transformation assay which has yielded transformation frequencies up to 10^{-3} transformants per recovered recipient. We confirm that T-DNA is transferred to yeast by a mechanism that mimics the transfer of T-DNA from *A. tumefaciens* to plant cells and not the conjugative transfer of the entire binary plasmid. Several pieces of evidence support the former hypothesis. *vir* gene induction is necessary for yeast transformation, as are T-DNA borders and T-DNA processing by VirD1/D2. Studies reported here and previously (14) show that *virB*- or *virD4*-mutant *A. tumefaciens* donors do not transform yeast, demonstrating a requirement for a transport apparatus for the T-DNA molecule. This present work has shown, however, that parallels with plant transformation are not complete. *A. tumefaciens* mutants blocked in attachment and subsequent transformation of plant cells are unaffected in T-DNA transformation of yeast.

The *A. tumefaciens*/plant genetic transfer system is a valuable tool in the field of plant biotechnology. However, limitations of this system to efficiently transform all plant species are, in part, a result of our lack of understanding of the mechanism of T-DNA transfer. Very little is known about the host factors that play a role in the transport, targeting, and chromosomal integration of the T-DNA. The present observation that *A. tumefaciens*-mediated T-DNA transfer to yeast parallels transfer to plants in many respects opens a new field of study that may provide insight into T-DNA transformation. Although direct comparisons between plants and yeast are premature at this point, studies indicate that some of their host mechanisms are similar. Janda and Ahlquist (43) demonstrated that yeast can support the replication of the brome mosaic virus (BMV) genome. Given that BMV is a plant virus suggests that yeast must contain cellular factors homologous to those in plants that allow the replication of higher eukaryotic viruses. Even more pertinent to the *A. tumefaciens*/yeast transformation system is the fact that the nuclear localization sequences of VirD2 function in yeast as well as in plants (44), suggesting that host components necessary for nuclear targeting of the T-DNA complex are present in both lower and higher eukaryotes. Future work will involve the identification of host factors involved in T-DNA transfer in yeast and the application of those observations to the *A. tumefaciens*/plant system.

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