Increased Agrobacterium-mediated transformation of Saccharomyces cerevisiae after deletion of the yeast ADA2 gene
Roushan, M.R., Shao, S., Poledri, I., Hooykaas, P.J.J., Heusden, G.P.H.

Citation
Roushan, M. R., S., S., P., I., H., P. J. J., H., G. P. H. (2021). Increased Agrobacterium-mediated transformation of Saccharomyces cerevisiae after deletion of the yeast ADA2 gene. Letters In Applied Microbiology, 1-10. doi:10.1111/lam.13605

Version: Publisher's Version
License: Creative Commons CC BY-NC-ND 4.0 license
Downloaded from: https://hdl.handle.net/1887/3243964

Note: To cite this publication please use the final published version (if applicable).
Significance and Impact of the Study: In this study, we have shown that deletion of ADA2, encoding a component of the ADA and SAGA transcriptional adaptor/histone acetyltransferase complexes, from the yeast *Saccharomyces cerevisiae* resulted in an increased efficiency of *Agrobacterium*-mediated transformation. This increased efficiency occurred irrespective of whether T-DNA integrates by homologous or non-homologous recombination. The effect on T-DNA integration by non-homologous recombination is of special importance as this process is very inefficient in *S. cerevisiae*. This result may open ways to improve transformation protocols for fungi and yeasts that are difficult to transform.

Abstract

*Agrobacterium tumefaciens* is the causative agent of crown gall disease and is widely used as a vector to create transgenic plants. Under laboratory conditions, the yeast *Saccharomyces cerevisiae* and other yeasts and fungi can also be transformed, and *Agrobacterium*-mediated transformation (AMT) is now considered the method of choice for genetic transformation of many fungi. Unlike plants, in *S. cerevisiae*, T-DNA is integrated preferentially by homologous recombination and integration by non-homologous recombination is very inefficient. Here we report that upon deletion of ADA2, encoding a component of the ADA and SAGA transcriptional adaptor/histone acetyltransferase complexes, the efficiency of AMT significantly increased regardless of whether integration of T-DNA was mediated by homologous or non-homologous recombination. This correlates with an increase in double-strand DNA breaks, the putative entry sites for T-DNA, in the genome of the ada2Δ deletion mutant, as visualized by the number of Rad52-GFP foci. Our observations may be useful to enhance the transformation of species that are difficult to transform.

Introduction

The soil pathogen *Agrobacterium tumefaciens* is renowned for its ability to transform a broad range of plant species (for review, see Nester et al. 1984; Tzfira and Citovsky 2006; Pacurar et al. 2011; Christie and Gordon 2014; Gelvin 2017). Under laboratory conditions, *Agrobacterium* can also transform the yeast *Saccharomyces cerevisiae* and many fungi (Bundock et al. 1995; Piers et al. 1996; de Groot et al. 1998; Bundock et al. 1999). This unique ability of *Agrobacterium* has made *Agrobacterium*-mediated transformation (AMT) not only essential for plant biology research, but also of increased importance for fungal research (for review, see Hooykaas et al. 2018). The DNA segment introduced into host cells by *Agrobacterium*, T-DNA, is derived from its tumour-inducing plasmid (Ti-plasmid). The T-DNA is transferred in a single-stranded form, the T-strand, which carries the VirD2 protein covalently linked at its 5'-end. The VirD2 protein contains a nuclear localization sequence which is
necessary for translocation of the T-strand into the nucleus of the host cell (Rossi et al. 1993). In the host cell nucleus, the T-strand is converted into a double-stranded T-DNA. Such T-DNA molecules can then circularize (Bundock et al. 1995) or form more complex extrachromosomal structures (Singer et al. 2012; Rolloos et al. 2014). They can be maintained if possessing a replicator (Bundock et al. 1995). More commonly, stable maintenance of T-DNA in the host cell is achieved after integration into the host genome. Host factors mediate T-DNA integration and this explains why T-DNA is integrated preferably by homologous recombination in yeast and by non-homologous recombination in plant cells (Offringa et al. 1990; Bundock et al. 1995).

Which host factors play a role in AMT is still far from clear. Importins from the host cell mediate import of the T-strand into the nucleus (Ballas and Citovsky 1997; Bhattcharjee et al. 2008). T-DNA integration is largely determined by the enzymes available in the host cells during infection. In yeast, enzymes important for T-DNA integration have been identified. Enzymes involved in homologous recombination (Rad51, Rad52) play an important role in T-DNA integration by homologous recombination in yeast (Bundock et al. 1995; Attikum and Hooykaas 2003; Ohmine et al. 2016), while enzymes involved in non-homologous end-joining (Yku70, Yku80, Lig4) are essential for T-DNA integration by non-homologous recombination in yeast (Bundock and Hooykaas 1996; van Attikum et al. 2001). In plants, however, the proteins involved in non-homologous end joining (NHEJ) are not essential for T-DNA integration, but transformants can only be obtained when Polymerase 0, which is absent from yeast and fungi, is available (van Kregten et al. 2016).

Several chromatin components or chromatin-modifying enzymes were identified which play a role in stable T-DNA transformation in plants, such as histone H2A (Mysore et al. 2000) and H3 (Anand et al. 2007) and the histone deacetylases HDT1 and HDT2 (Crane and Gelvin 2007). In yeast histone, acetyltransferases (Gcn5, Ngg1, Ya1 and Eal7) and deacetylases (Hst4, Hda2 and Hda3), involved in chromatin modification, have also been identified as factors affecting AMT (Soltani et al. 2009). ARP6 encoding an actin-related protein that is part of the SWR1 chromatin remodelling complex, negatively regulates AMT (Luo et al. 2015). Preliminary results in our group demonstrated that deletion of ADA2 leads to enhanced transformation, when using a T-DNA that integrates by homologous recombination (Soltani 2009). The Ada2 protein is the chromatin-binding subunit of the SAGA (Spt–Ada–Gcn5 acetyltransferase) histone acetyltransferase (HAT) complex. This complex is involved in the post-translational modifications of histones that are crucial for chromatin-dependent functions and the regulation of numerous cellular processes in response to environmental cues (Sternet et al. 2002; for recent review, see Soffers and Workman 2020). Ada2 can interact with Gcn5 directly to increase its HAT activity which preferentially acetylates histone H3 and histone H2B (Grant et al. 1997; Hoke et al. 2008). The Spt constituent of the SAGA complex, consisting of the proteins Spt3 and Spt20, has a coactivator role in the recruitment of TATA-binding protein (Dudley et al. 1999). Ada2 is evolutionarily conserved among eukaryotes and has been described in several organisms, including Arabidopsis (Hark et al. 2009) and Drosophila (Muratoglu et al. 2003). In Arabidopsis, the orthologues of Ada2 physically associate with Gcn5 and enhance its HAT activity to regulate gene expression under environmental stress conditions such as cold, drought and salt stress (Hark et al. 2009). In 2009, an additional function of Ada2, independent of Gcn5, was identified in yeast. The novel role of Ada2 was to promote transcriptional silencing at telomeres through binding to Sir2 and to prevent the inward spread of heterochromatin regions (Jacobson and Pillus 2009).

In the present study, we investigated the role of ADA2 in AMT in more detail. To this end, we analysed the effect of deletion of ADA2 on T-DNA integration by homologous and non-homologous recombination and showed that deletion of ADA2 resulted in an increased transformation efficiency for both targeted and random T-DNA integration.

Results and discussion

Increased AMT in yeast ada2Δ deletion mutants using T-DNA allowing integration by homologous recombination

Our preliminary results with the diploid yeast strain BY4743 suggested that the efficiency of AMT is increased in the ada2Δ deletion mutant (Soltani 2009). To investigate whether this is also the case in the isogenic haploid BY4741, we constructed an ada2Δ deletion mutant in BY4741 by replacing the ADA2 coding sequence by an hygromycin resistance marker. Subsequently, the strain was transformed with the Agrobacterium strain LBA1100 carrying binary vector pRAL7100 allowing integration of URA3 into the chromosomal PDA1 locus by homologous recombination (Fig. 1a) (Bundock et al. 1995). As shown in Fig. 1a, the BY4741 ada2Δ deletion mutant had a four-fold increased transformation efficiency compared to the parental strain at frequencies of 4.4 ± 0.6 × 10⁻⁴ and 1.1 ± 0.4 × 10⁻⁴ (mean ± SEM, n = 3, P = 0.01), respectively.

For our further studies, we preferred to use a marker-free ada2Δ mutant, as this would enable the use of a
lager range of vectors for transformation. To this end, we deleted the ADA2 coding region in BY4741 using the CRISPR-Cas technology resulting in strain ada2ΔMF. This strain and BY4741 were co-cultivated with Agrobacterium strain EHA105 carrying plasmid pSDM8001 containing the KanMX cassette flanked by sequences allowing integration into the PDA1 locus (Fig. 1b). G418-resistant transformants were selected and the transformation frequency was calculated. As shown in Fig. 1b, the transformation frequency of this ADA2 deletion mutant was increased (3.1-fold) as well (9.7 ± 2.5 × 10^{-5} vs 3.1 ± 0.6 × 10^{-5}, mean ± SEM, n = 5, P = 0.03).

Increased AMT in yeast ada2Δ deletion mutants using T-DNA lacking sequences homologous to the yeast genome

Although homologous recombination is the predominant mechanism of T-DNA integration in yeast, integration via NHEJ is possible as well (Bundock and Hooykaas 1996; van Attikum et al. 2001). To investigate the effect of the ada2Δ deletion on T-DNA integration via NHEJ, we exploited Agrobacterium strain EHA105 harbouring plasmid pSDM8000. This plasmid contains a T-DNA with the KanMX cassette, but lacks homology with the BY4741 genome and has no yeast replication origin (Fig. 2a). As illustrated in Fig. 2a, Agrobacterium carrying pSDM8000 is able to transform BY4741, but at an extremely low frequency of 5 ± 2 × 10^{-6} (mean ± SEM, n = 10). Compared to the wild-type strain, the transformation efficiency for the ada2ΔMF deletion mutant was significantly (P = 0.008) higher (23 ± 6 × 10^{-6}, mean ± SEM, n = 10). As shown in Fig. 2b, addition of a wild-type copy of ADA2 on the centromeric plasmid pRS315 to the ada2ΔMF deletion mutant restored the low wild-type transformation efficiency, indicating that the enhanced transformation of the ada2ΔMF deletion mutant was due to the ADA2 deletion and not to off-target effects of the CRISPR-Cas method. An additional copy of ADA2 does not result in a further decrease in the transformation efficiency of BY4741.

To check whether the T-DNA is integrated into the yeast chromosome in the ada2ΔMF strain and not present as an extrachromosomal structure, Southern blot analysis was done. To this end, the genomic DNA was isolated from 12 independent transformants obtained after co-cultivation of ada2ΔMF or BY4741 with Agrobacterium strain EHA105 carrying pSDM8000. The DNA was digested with EcoRV and then hybridized to a probe containing the KanMX cassette. As pSDM8000 contains only two EcoRV sites outside the T-DNA region, the non-integrated plasmid and the integrated total plasmid will...
yield a 8·4 kb fragment hybridizing to the KanMX probe. Circularization of T-DNA will yield a circular DNA lacking EcoRV sites of approximately 1·5 kb. On the other hand, upon integration of T-DNA, the size of the chromosomal fragment containing the T-DNA and thus hybridizing to the KanMX probe is predicted to vary as it is dependent on the local presence of EcoRV sites in the genome. When integration occurs by NHEJ, it is expected that integration occurs at a variety of chromosomal positions and in different transformants fragments of different sizes are expected to hybridize to the probe. As shown in Fig. 2c, the probe indeed hybridizes to different EcoRV fragments in the DNA from the different transformants. For one of the transformants, hybridization to a fragment of about 1 kb which is smaller than the T-DNA (c. 1·5 kb) was found (Fig. 2c, lane 2), possibly caused by integration of a part of the T-DNA. No hybridization was found for DNA isolated from the untransformed ada2ΔMF strain (Fig. 2c, lane 13). These results indicate that the T-DNA was integrated at different sites into the chromosomal DNA of the transformants and therefore likely had occurred by NHEJ.

Increased double-strand breaks in the ada2Δ deletion mutant
Ada2-dependent histone acetylation has been shown to be involved in double-strand break repair (Muñoz-Galván...
As double-strand breaks can promote T-DNA integration, we investigated the sensitivity of the ada2Δ deletion mutant for DNA damaging agents. The DNA alkylating agent methyl methane sulfonate (MMS) induces double-strand breaks during replication and hydroxyurea (HU) is a potent inhibitor of the enzyme ribonucleotide reductase in S-phase and leads to stalling of DNA replication. Survival viability was estimated by plating serial dilutions of cultures of wild type and ada2Δ deletion mutant cells on YPD plates containing MMS or HU. Yeast cells were serially diluted and spotted onto the plates. The photos were taken after 3 days and representative results of three independent experiments are shown. (b) The Rad52 protein was marked by GFP to visualize double-strand breaks in ada2Δ cells. The DNA repair foci were observed using confocal microscopy. (c) The percentage of cells showing DNA repair foci is shown for both BY4741 and the ada2Δ deletion mutant carrying pRS315 or pRS315[ADA2]. The percentage of cells with foci is the average with SEM of the percentages determined after seven independent cultivations. For each strain at least 2100 cells were observed.

Figure 3 The ada2Δ deletion mutant is more sensitive to the DNA damaging agents methyl methane sulfonate (MMS) and hydroxyurea (HU) and has an increased number of double-strand breaks. (a) Both wild-type BY4741 and ada2Δ deletion mutant were tested on yeast extract-peptone-dextrose (YPD) plates with two commonly used DNA damaging agents, MMS and HU. Yeast cells were serially diluted and spotted onto the plates. (b) The Rad52 protein was marked by GFP to visualize double-strand breaks in ada2Δ cells. The DNA repair foci were observed using confocal microscopy. (c) The percentage of cells showing DNA repair foci is shown for both BY4741 and the ada2Δ deletion mutant carrying pRS315 or pRS315[ADA2]. The percentage of cells with foci is the average with SEM of the percentages determined after seven independent cultivations. For each strain at least 2100 cells were observed.
Table 1 Plasmids used in this study

| Plasmid | Specifications | Source/Reference |
|---------|---------------|-----------------|
| pAG32  | Plasmid with the hph gene encoding hygromycin B phosphotransferase | Goldstein and McCusker (1999) |
| pYMD27 | Plasmid used as PCR template for C-terminal eGFP tagging using the kanMX marker | Janke et al. (2004) |
| pML104 | Plasmid for expression of Ca9 and contains guide RNA expression cassette. | John Wyrick (Addgene plasmid # 67638) |
| pML104 [ADA2 disr] (pSDM3793) | Plasmid for deletion of ADA2 by the CRISPR-Cas technology |  |
| pML104 [ADA2] (pSDM3792) | Yeast episomal cloning vector with a URA3 marker | Carlson and Botstein (1982) |
| pRS315 | Yeast centromeric plasmid with a LEU2 marker | Sikorski and Hieter (1989) |
| pRS315 [ADA2] (pSDM3792) | pRS315 containing a 2026 bp fragment containing ADA2 under control of its native promoter and terminator | This study |
| pRAL7100 | Agrobacterium binary vector with URA3 selectable marker and PDA1 flanking sequence | Bundock et al. (1995) |
| pSDM8000 | Agrobacterium binary vector with kanMX selectable marker | Attikum and Hooykaas (2003) |
| pSDM8001 | Agrobacterium binary vector with kanMX selectable marker and PDA1 flanking sequence | Attikum and Hooykaas (2003) |
| pUG6  | Plasmid containing the KanMX gene disruption cassette | Guldener et al. (1996) |

regulated by ADA2. The expression of approximately 2.5% of all yeast genes was found to be affected at least twofold in an ada2Δ deletion mutant (Hoke et al. 2008). It can be speculated that the increased AMT efficiency of the ada2Δ deletion mutant could be the consequence or an indirect influence caused by altered regulation of certain genes involved in the integration process. Besides such indirect roles of ADA2 in gene expression or chromatin structure, we showed there is more DNA damage in an ada2Δ deletion mutant as revealed by an increased number of Rad52 foci (Fig. 3). Our results are in line with the results obtained in a large-scale screen for deletion mutants with an increased percentage of cells with one or more spontaneous Rad52-YFP foci (Alvaro et al. 2007). In the initial screen, 11% of the ada2Δ cells contained foci, compared to 5% of the wild-type cells. Furthermore, it has been shown that an Ada2 homologue in plants plays a role in double-strand break repair (Lai et al. 2018). Due to the absence of ADA2, there may be either more DNA damaging events in the cell or this damage may be repaired less efficiently, thus providing more available sites for T-DNA integration.

AMT of some yeasts and fungi occurs only with a very low efficiency (Idnurm et al. 2017). To improve this efficiency, it may be worthwhile to investigate whether disruption of ADA2 homologues in such organisms has a similar effect as we observed in the yeast S. cerevisiae. As disruption of ADA2 has additional effects, the use of a system that temporarily represses ADA2 expression may be of interest.

Materials and methods

Agrobacterium strains and growth conditions

Agrobacterium tumefaciens strains LBA1100 (Beijersbergen et al. 1992) and EHA105 (Hood et al. 1993) were used; both strains are derivatives of strain C58, in which the natural Ti plasmid is replaced by a vir helper plasmid. Binary vector plasmids were introduced into Agrobacterium by electroporation (Dulk-Ras and Hooykaas 1995). Agrobacterium tumefaciens strains were grown and maintained at 28°C in LC medium (10 g l⁻¹ tryptone, 5 g l⁻¹ yeast extract and 8 g l⁻¹ NaCl) containing carbenicillin (100 µg ml⁻¹), kanamycin (100 µg ml⁻¹) or rifampicin (20 µg ml⁻¹) when required.

Yeast strains and growth conditions

Saccharomyces cerevisiae strains used in this study are BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) (Brachmann et al. 1998), adaΔ (BY1135) (BY4741 ada2Δ::hphMX4) (this study), ada2ΔΔMF (BY1148) (BY4741 ada2Δ) (this study), BY4741 Rad52-GFP (BY1137) (BY4741 RAD52-GFP-kanMX4) (this study) and ada2Δ Rad52-GFP (BY1138) (BY4741 ada2::hphMX4 RAD52-GFP-kanMX4) (this study). Plasmids used in this study are shown in Table 1 and primers in Table 2. The ada2Δ deletion in BY4741 containing the HphMX4 cassette was constructed using the PCR-mediated one-step gene disruption method. The disruption cassette was obtained by PCR with the Ada2-Fw and Ada2-Rev primers using pAG32 as template and hygromycin-resistant transformants were selected. Correct integration was checked by PCR using the primers Ada2-ctrl-Fw and Ada2-ctrl-Rev. For C-terminal labelling of Rad52 with GFP, a DNA fragment was used obtained by PCR with the primers Rad52-gfp-Fw and Rad52-gfp-Rev and plasmid pYM27 as template.
Table 2 Primers used in this study

| Primer                | Sequence                              |
|----------------------|---------------------------------------|
| Ada2-fw              | TAAAATACGCTAGTCGAAATATATACATTAAGCAAAAAAGACGCTGAAGCTTCGTACGC |
| Ada2-Rev             | ATATAAATTGAGCAGAATGATGTACGTTTCAATTTTTTTTTTGGCCGGCCGCATAGGCCAC |
| Ada2-Ctrl-fw         | AGCACCTCAGGAACAAGCGGCAGCTGACGTTGACG |
| Ada2-Ctrl-Rev        | GGTCCCTTTATGACTTGGCCGCTTTGCTTTATCGAG |
| Rad52-gfp-fw         | AGAGAAATGGGCAAGGACCAAAATCCTCTGAGCAAGCAGCTGACGTTGACG |
| Rad52-gfp-Rev        | AGTAATAAATTGAGCAGAATGATGTACGTTTCAATTTTTTTTTTGGCCGGCCGCATAGGCCAC |
| Ada2-repair-1        | ACCCTCAGTTTCGATATATACATTAAGCAAAAAGACAGCTGAAGCTTCGTACGC |
| Ada2-repair-2        | AAAAGAACTCAAAATGACACATCTAGTTATTATTGGCCAAGTCATAAA |
| P-gRNA-5             | GGAGAAGTTGGAAGACCAAAGATCAATCCCATGCAGCAAGCCTACTCGTACGCTGCAGGTCGAC |
| Ada2-guide-Rev       | CTAGCTCTCAATATACTAGTACATTTTCAATTTTTTTTTTGGCCGGCCGCATAGGCCAC |
| HindIII-ADA2-Fw      | AAAAAGCTTTTTTATCTGCTTTTTTCTTTATCTTTATTC |
| HindIII-ADA2-Rv      | AAAAAGCTTTTTTATCTGCTTTTTTCTTTATCTTTATTC |
| PstI-ADA2-Rv         | AAAAAGCTTTTTTATCTGCTTTTTTCTTTATCTTTATTC |
| PstI-ADA2-fw         | AAAAAGCTTTTTTATCTGCTTTTTTCTTTATCTTTATTC |
| KANMX-fw             | GGTCCCTTTATGACTTGGCCGCTTTGCTTTATCGAG |
| KANMX-Rev            | GGTCCCTTTATGACTTGGCCGCTTTGCTTTATCGAG |

For the marker-free deletion of ADA2 by the CRISPR-Cas technique, BY4741 was co-transformed with 250 ng of plasmid pML104[ADA2 disr] and 1 μg of the repair fragment and transformants were selected for uracil prototrophy. The repair fragment was obtained by annealing oligos Ada2-repair-1 and Ada2-repair-2. Transformants are expected to have the required deletion but they still contain the pML104[ADA2 disr] plasmid. The transformants were streaked on a plate containing 5-fluoro-orotic acid (1 mg ml⁻¹) and uracil in addition to methionine, histidine and leucine to select for cells that had lost the plasmid. After incubation for 5 days at 30°C, colonies were analysed by restreaking on plates containing or lacking uracil. Uracil auxotrophic transformants were selected and DNA was isolated. The ADA2 deletion was analysed by PCR using primers Ada2-ctrl-Fw and Ada2-ctrl-Rev, followed by sequencing of the PCR fragments.

The PCR products and plasmids were transferred to yeast cells using the lithium–acetate transformation protocol (Gietz et al. 1995). Yeast was grown at 30°C in yeast extract–peptone–dextrose (YPD) medium, supplemented when required, with the appropriate antibiotic G418 (200 μg ml⁻¹) or hygromycin (200 μg ml⁻¹) or in selective minimal yeast (MY) medium (Zonneveld 1986) supplemented with appropriate nutrients. For spot plate assays, cultures were adjusted to an OD₆₀₀ of 0.1 after growth to saturation in liquid YPD. Then, 10-fold serial dilutions were made and aliquots of 5 μl were spotted on YPD, on YPD containing 5 μg ml⁻¹ HU and on YPD containing 5 μg ml⁻¹ MMS.

Plasmid construction

The centromeric plasmid pRS315[ADA2] was constructed by ligation of a 2026 bp PCR fragment containing the ADA2 promoter, coding and terminator sequences into pRS315 after digestion with HindIII and PstI. The PCR fragment was obtained using the primers HindIII-ADA2-Fw and PstI-ADA2-Rv and BY4741 genomic DNA as template.

For the marker-free deletion of ADA2, the CRISPR-Cas technology was used. Plasmid pML104 was digested with SacII and SwaI and the digested vector was isolated by gel electrophoresis. The guide RNA fragment was obtained by PCR on undigested pML104 using primers P-gRNA-5 and Ada2-guide-Rev. The guide fragment was digested with SacII and ligated in pML104 digested with SacII and SwaI yielding plasmid pML104[ADA2 disr].

Agrobacterium-mediated transformation efficiency test

AMT efficiency was determined as described (Bundock et al. 1995) with some modifications. First, S. cerevisiae strains and Agrobacterium were cultured overnight at 30 and 28°C, respectively, under continuous agitation and with the appropriate nutrition or antibiotic selection. The following day, the Agrobacterium cells were washed and re-suspended to an OD₆₀₀ of 0.25 in induction medium (IM) with added glucose (10 mmol l⁻¹), acetosyringone (0.2 mmol l⁻¹) and the appropriate antibiotics, and incubated for another 6 h at 28°C. Meanwhile, yeast cultures were diluted to an OD₆₀₀ of 0.1 and incubated in either liquid YPD or MY (when the yeast contained a plasmid) medium. After 6 h, the yeast cells were washed and re-suspended in 0.5 ml of IM, to a final OD₆₀₀ of 0.4–0.6 and mixed with an equal volume of Agrobacterium cells and vigorously vortexed. Subsequently, 100 μl of the mixture was pipetted onto sterile nitrocellulose filters laid on 1 M plates supplemented with histidine, leucine and methionine. Once filters were dry, plates were incubated at 21°C for 6–7 days. After co-cultivation, the cells on each filter were resuspended and then spread onto solid plates
medium containing cefotaxime (200 μg ml⁻¹) with or without G418 (200 μg ml⁻¹). Finally, after a 3-day incubation at 30°C, colonies were counted. Yeast AMT efficiency was calculated by dividing the number of colonies on the selective plate by the number of colonies on the non-selective plates.

Southern blotting
Two micrograms of isolated yeast DNA were digested with EcoRV. The digested DNA was separated on a 0.7% (w/v) agarose gel and transferred onto a Nylon membrane (positively charged; Roche, Mannheim, Germany) by capillary blotting. The blot was hybridized to a KanMX probe at 68°C, labelled using the PCR DIG Labeling Mix (Roche), pUG6 as template and the primers KANMX-Fw and KANMX-Rev. Bound probe was detected using antidigoxigenin-alkaline phosphatase and the CDP-star substrate (Roche).

Confocal microscopy
For microscopy, a Zeiss Axioscan confocal microscope with a 63× oil objective was used. GFP was detected using an argon laser of 488 nm and a band-pass emission filter with 505–600 nm. Images were processed with ImageJ (ImageJ National Institute of Health) (Schindelin et al. 2012).

Acknowledgements
This work was partly supported by the China Scholarship Council (CSC), grant number 201406740044 and partly by the fellowship associated with the appointment of PJHH as Academy Professor by the Royal Dutch Academy of Sciences.

Conflict of Interest
The authors declare that they have no conflict of interest.

Authors’ contributions
M.R.R. and S.S. performed the experiments and contributed to the design of the study and writing the manuscript. I.P. performed the experiments and participated in data analysis. P.J.J.H. contributed to the design of the study and writing the manuscript and provided the funding support. G.P.H.v.H. supervised the study and contributed to the design of the study and writing the manuscript. All authors read and approved the submission of the final manuscript.

Data Availability Statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

References
Alvaro, D., Lisby, M. and Rothstein, R. (2007) Genome-wide analysis of Rad52 foci reveals diverse mechanisms impacting recombination. PloS Genet 3, e228.
Anand, A., Vaghchhipawala, Z., Ryu, C.M., Kang, L., Wang, K., del-Pozo, O., Martin, G.B. and Mysore, K.S. (2007) Identification and characterization of plant genes involved in Agrobacterium-mediated plant transformation by virus-induced gene silencing. Mol Plant Microbe Interact 20, 41–52.
van Attikum, H., Bundock, P. and Hooykaas, P.J.J. (2001) Non-homologous end-joining proteins are required for Agrobacterium T-DNA integration. EMBO J 20, 6550–6558.
van Attikum, H. and Hooykaas, P.J.J. (2003) Genetic requirements for the targeted integration of Agrobacterium T-DNA in Saccharomyces cerevisiae. Nucl Acid Res 31, 826–832.
Balasubramanian, R., Pray-Grant, M.G., Selleck, W., Grant, P.A. and Tan, S. (2002) Role of the Ada2 and Ada3 transcriptional coactivators in histone acetylation. J Biol Chem 277, 7789–7795.
Ballas, N. and Citovsky, V. (1997) Nuclear localization signal binding protein from Arabidopsis mediates nuclear import of Agrobacterium VirD2 protein. Proc Natl Acad Sci USA 94, 10723–10728.
Beijersbergen, A., Den Dulk-Ras, A., Schilperoort, R.A. and Hooykaas, P.J.J. (1992) Conjugative transfer by the virulence system of Agrobacterium tumefaciens. Science 256, 1324–1327.
Berger, S.I., Piña, B., Silverman, N., Marcus, G.A., Agapite, J., Regier, J.L., Triezenberg, S.J. and Guarente, L. (1992) Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. Cell 70, 251–265.
Bhattacharjee, S., Lee, L.Y., Oltmanns, H., Cao, H., Veena, C.J. and Gelvin, S.B. (2008) Atlmpa-4, an Arabidopsis importin α isoform, is preferentially involved in Agrobacterium mediated plant transformation. Plant Cell 20, 2661–2680.
Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P. and Boeke, J.D. (1998) Designer deletion strains derived from Saccharomyces cerevisiae S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. Yeast 14, 115–132.
Bundock, P., den Dulk-Ras, A., Beijersbergen, A. and Hooykaas, P.J.J. (1995) Trans-kingdom T-DNA transfer from Agrobacterium tumefaciens to Saccharomyces cerevisiae. EMBO J 14, 3206–3214.
Bundock, P. and Hooykaas, P.J.J. (1996) Integration of Agrobacterium tumefaciens T-DNA in the Saccharomyces
cerevisiae genome by illegitimate recombination. Proc Natl Acad Sci USA 93, 15272–15275.
Bundock, P., Mrózcek, K., Winkler, A.A., Steensma, H.Y. and Hoooykaas, P.J.J. (1999) T-DNA from Agrobacterium tumefaciens as an efficient tool for gene targeting in Kluyveromyces lactis. Mol Gen Genet 261, 115–121.
Carlson, M. and Botstein, D. (1982) Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell 28, 145–154.
Christie, P.J. and Gordon, J.E. (2014) The Agrobacterium Ti plasmids. Microbiology Spectrum 2, 1–18.
Crane, Y.M. and Gelvin, S.B. (2007) RNAi-mediated gene silencing reveals involvement of Arabidopsis chromatin-related genes in Agrobacterium-mediated root transformation. Proc Natl Acad Sci USA 104, 15156–15161.
Dudley, A.M., Rougeulle, C. and Winston, F. (1999) The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo. Genes Dev 13, 2940–2945.
den Dulk-Ras, A. and Hoooykaas, P.J.J. (1995) Electroporation of Agrobacterium tumefaciens. In Plant Cell Electroporation and Electrofusion Protocols ed. Nickoloff, J.A. pp. 63–72. Totowa, NJ: Springer.
Gelvin, S.B. (2017) Integration of Agrobacterium T-DNA into the plant genome. Annu Rev Genet 51, 195–217.
Gietz, R.D., Schiestl, R.H., Willems, A.R. and Woods, R.A. (1995) Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast 11, 355–360.
Goldstein, A.L. and McCusker, J.H. (1999) Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Yeast 15, 1541–1553.
Grant, P.A., Duggan, L., Côté, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T. et al. (1997) Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev 11, 1640–1650.
de Groot, M.J., Bundock, P., Hoooykaas, P.J.J. and Beijersbergen, A.G. (1998) Agrobacterium tumefaciens-mediated transformation of filamentous fungi. Nat Biotechnol 16, 839–842.
Güldener, U., Heck, S., Fiedler, T., Beinhauer, J. and Hegemann, J.H. (1996) A new efficient gene disruption cassette for repeated use in budding yeast. Nucl Acid Res 24, 2519–2524.
Hark, A.T., Vlachonasios, K.E., Pavangadkar, K.A., Rao, S., Gordon, H., Adamakis, I.D., Kaldis, A., Thomashow, M.F. et al. (2009) Two Arabidopsis orthologs of the transcriptional coactivator ADA2 have distinct biological functions. Biochim Biophys Acta-Gene Regul Mech 1789, 117–124.
Hoke, S.M., Genereaux, J., Liang, G. and Brandl, C.J. (2008) A conserved central region of yeast Ada2 regulates the histone acetyltransferase activity of Gcn5 and interacts with phospholipids. J Mol Biol 384, 743–755.
Hood, E.E., Gelvin, S.B., Melchers, L.S. and Hoekema, A. (1993) New Agrobacterium helper plasmids for gene transfer to plants. Transgenic Res 2, 208–218.
Hoooykaas, P.J.J., van Heusden, G.P.H., Niu, X., Roushan, M.R., Soltani, J., Zhang, X. and van der Zaal, B.J. (2018) Agrobacterium-mediated transformation of yeast and fungi. Curr Top Microbiol Immunol 418, 349–374.
Ishimura, A., Bailey, A.M., Cairns, T.C., Elliott, C.E., Foster, G.D., Ianieri, G. and Jeon, J. (2017) A silver bullet in a golden age of functional genomics: the impact of Agrobacterium-mediated transformation of fungi. Fungal Biol Biotechnol 4, 6.
Jacobson, S. and Pillus, L. (2009) The SAGA subunit Ada2 functions in transcriptional silencing. Mol Cell Biol 29, 6033–6045.
Janke, C., Magiera, M.M., Rathfelder, N., Taxis, C., Reber, S., Maekawa, H., Moreno-Borchart, A., Doenges, G. et al. (2004) A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. Yeast 21, 947–962.
van Kregten, M., de Pater, S., Romeijn, R., van Schendel, R., Hoooykaas, P.J.J. and Tijsterman, M. (2016) T-DNA integration in plants results from polymerase-0-mediated DNA repair. Nature Plants 2, 16164.
Lai, J., Jiang, J., Wu, Q., Mao, N., Han, D., Hu, H. and Yang, C. (2018) The transcriptional coactivator ADA2b recruits a structural maintenance protein to double-strand breaks during DNA repair in plants. Plant Physiol 176, 2613–2622.
Laughery, M., Hunter, T., Brown, A., Hoopes, I., Ostbye, T., Shumaker, T. and Wyrick, J.J. (2015) New vectors for simple and streamlined CRISPR–Cas9 genome editing in Saccharomyces cerevisiae. Yeast 32, 711–720.
Lisby, M., Rothstein, R. and Mortensen, U.H. (2001) Rad52 forms DNA repair and recombination centers during S phase. Proc Natl Acad Sci USA 98, 8276–8282.
Luo, Y., Chen, Z., Zhu, D., Tu, H. and Pan, S.Q. (2015) Yeast actin-related protein Arb6 negatively regulates Agrobacterium-mediated transformation of yeast cell. Bio Med Res Int 2015, 275902.
Magori, S. and Citovsky, V. (2011) Epigenetic control of Agrobacterium T-DNA integration. Biochim Biophys Acta - Gene Regul Mech 1809, 388–394.
Muñoz-Galván, S., Jimeno, S., Rothstein, R. and Aguiler, A. (2013) Histone H3K56 acetylation, Rad52, and non-DNA repair factors control double-strand break repair choice with the sister chromatid. PLoS Genet 9, e1003237.
Muratoglu, S., Georgieva, S., Papai, G., Scheer, E., Enunlu, I., Komonyi, O., Cserpan, I., Lebedeva, L. et al. (2003) Two different Drosophila ADA2 homologues are present in distinct GCN5 histone acetyltransferase-containing complexes. Mol Cell Biol 23, 306–321.
Mysoore, K.S., Nam, J. and Gelvin, S.B. (2000) An Arabidopsis histone H2A mutant is deficient in Agrobacterium T-DNA integration. Proc Natl Acad Sci USA 97, 948–953.
Transformation of yeast ada2 mutant

M.R. Roushan et al.

Nester, E.W., Gordon, M.P., Amasino, R.M. and Yanofsky, M.F. (1984) Crown gall: a molecular and physiological analysis. *Annu Rev Plant Physiol* **35**, 387–413.

Offringa, R., de Groot, M.J., Haagsman, H.J., Does, M.P., van den Elzen, P.J. and Hooykaas, P.J.J. (1990) Extrachromosomal homologous recombination and gene targeting in plant cells after *Agrobacterium* mediated transformation. *EMBO J* **9**, 3077–3084.

Ohmine, Y., Satoh, Y., Kiyokawa, K., Yamamoto, S., Moriguchi, K. and Suzuki, K. (2016) DNA repair genes *RAD52* and *SRS2*, a cell wall synthesis regulator gene *SMI1*, and the membrane sterol synthesis scaffold gene *ERG28* are important in efficient *Agrobacterium*-mediated yeast transformation with chromosomal T-DNA. *BMC Microbiol* **16**, 58.

Păcurar, D.I., Thordal-Christensen, H., Păcurar, M.L., Pamfil, D., Botez, C. and Bellini, C. (2011) *Agrobacterium tumefaciens*: From crown gall tumors to genetic transformation. *Physiol Mol Plant Pathol* **76**, 76–81.

Piers, K.L., Heath, J.D., Liang, X., Stephens, K.M. and Nester, E.W. (1996) *Agrobacterium tumefaciens*-mediated transformation of yeast. *Proc Natl Acad Sci USA* **93**, 1613–1618.

Rolloos, M., Dohmen, M.H., Hooykaas, P.J.J. and van der Zaal, B.J. (2014) Involvement of Rad52 in T-DNA circle formation during *Agrobacterium* tumefaciens-mediated transformation of *Saccharomyces cerevisiae*. *Mol Microbiol* **91**, 1240–1251.

Rossi, L., Hohn, B. and Tinland, B. (1993) The VirD2 protein of *Agrobacterium tumefaciens* carries nuclear localization signals important for transfer of T-DNA to plant. *Mol Gen Genet* **239**, 345–353.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C. et al. (2012) Fiji: an open-source platform for biological-image analysis. *Nat Methods* **9**, 676–682.

Shilo, S., Tripathi, P., Melamed-Bessudo, C., Tzfadia, O., Muth, T.R. and Levy, A.A. (2017) T-DNA-genome junctions form early after infection and are influenced by the chromatin state of the host genome. *PLoS Genet* **13**, e1006875.

Sikorski, R.S. and Hieter, P. (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**, 19–27.

Singer, K., Shibolet, Y.M., Li, J. and Tzfira, T. (2012) Formation of complex extrachromosomal T-DNA structures in *Agrobacterium*-infected plants. *Plant Physiol* **160**, 511–522.

Soffers, J. and Workman, J.L. (2020) The SAGA chromatin-modifying complex: the sum of its parts is greater than the whole. *Genes Dev* **34**, 1287–1303.

Soltani, J. (2009) *Host genes involved in Agrobacterium-mediated transformation*. PhD Thesis, Leiden University, Leiden, The Netherlands.

Soltani, J., van Heusden, G.P.H. and Hooykaas, P.J.J. (2009) Deletion of host histone acetyltransferases and deacetylases strongly affects *Agrobacterium*-mediated transformation of *Saccharomyces cerevisiae*. *FEMS Microbiol Lett* **298**, 228–233.

Sterner, D.E., Wang, X., Bloom, M.H., Simon, G.M. and Berger, S.L. (2002) The SANT domain of Ada2 is required for normal acetylation of histones by the yeast SAGA complex. *J Biol Chem* **277**, 8178–8186.

Tzfira, T. and Citovsky, V. (2006) *Agrobacterium*-mediated genetic transformation of plants: biology and biotechnology. *Curr Opin Biotechnol* **17**, 147–154.

Zonneveld, B.J.M. (1986) Cheap and simple yeast media. *J Microb Meth* **4**, 287–291.