

RESEARCH ARTICLE

Open Access

Inverted terminal repeats of adeno-associated virus decrease random integration of a gene targeting fragment in *Saccharomyces cerevisiae*

Alvaro Galli* and Tiziana Cervelli*

Abstract

Background: Homologous recombination mediated gene targeting is still too inefficient to be applied extensively in genomics and gene therapy. Although sequence-specific nucleases could greatly stimulate gene targeting efficiency, the off-target cleavage sites of these nucleases highlighted the risk of this strategy. Adeno-associated virus (AAV)-based vectors are used for specific gene knockouts, since several studies indicate that these vectors are able to induce site-specific genome alterations at high frequency. Since each targeted event is accompanied by at least ten random integration events, increasing our knowledge regarding the mechanisms behind these events is necessary in order to understand the potential of AAV-mediated gene targeting for therapy application. Moreover, the role of AAV regulatory proteins (Rep) and inverted terminal repeated sequences (ITRs) in random and homologous integration is not completely known. In this study, we used the yeast *Saccharomyces cerevisiae* as a genetic model system to evaluate whether the presence of ITRs in the integrating plasmid has an effect on gene targeting and random integration.

Results: We have shown that the presence of ITRs flanking a gene targeting vector containing homology to its genomic target decreased the frequency of random integration, leading to an increase in the gene targeting/random integration ratio. On the other hand, the expression of Rep proteins, which produce a nick in the ITR, significantly increased non-homologous integration of a DNA fragment sharing no homology to the genome, but had no effect on gene targeting or random integration when the DNA fragment shared homology with the genome. Molecular analysis showed that ITRs are frequently conserved in the random integrants, and that they induce rearrangements.

Conclusions: Our results indicate that ITRs may be a useful tool for decreasing random integration, and consequently favor homologous gene targeting.

Keywords: Yeast, AAV, ITRs, Homologous recombination, Random integration

Background

Gene targeting is the process by which exogenously delivered DNA is used to modify a genomic target by homologous recombination. Since in human cells the spontaneous frequency of homologous recombination is on the order of 10^{-6} , the gene targeting approach is not efficient enough to be applied for clinical use [1]. However, several reports indicate that sequence-specific nucleases, which induce a site-specific double strand

break in the target DNA, can increase gene targeting efficiency up to 50,000-fold [2]; this approach is thus particularly useful for manipulating primary human cells with therapeutic potential [3,4]. Although there has been considerable excitement about the potential application of zinc finger nucleases, since these enzymes allow highly specific, targeted genome modification in live cells [5], several reports show the off-target cleavage sites of these nucleases, highlighting the risk of using this approach [6,7]. Therefore, the search for new tools to improve the efficiency of gene targeting is still very important. Recently, the induction of a site-specific single-strand nick significantly increased gene targeting

* Correspondence: alvaro.galli@ifc.cnr.it; tiziana.cervelli@ifc.cnr.it
Yeast Genetics and Genomics Group, Institute of Clinical Physiology, CNR, via
Moruzzi 1, 56125 Pisa, Italy

[8]. Our previously published studies show that the over-expression or nuclear permeation of the *Saccharomyces cerevisiae* protein Rad52 strongly increases homologous recombination and gene targeting in HeLa cells [9,10]. An alternative method of homologous recombination mediated gene targeting consists of exploiting the recombinogenic nature of the adeno-associated virus (AAV) vector genome [11]. The AAV single stranded (ss) DNA genome contains two overlapping open reading frames flanked by two inverted repeated sequences (ITRs), the only elements required in *cis* for replication and integration. The Rep open reading frame codes for Rep proteins essential for DNA replication, integration and packaging. The Cap codes for the proteins essential for capsid formation [12]. Vectors based on AAV, which deliver single-stranded, linear DNA genomes, are able to efficiently introduce many types of mutations into homologous target loci at a frequency approaching 1% in mammalian cells, and are currently used as gene targeting vectors [13-16]. However, using this method, each homologous targeted event occurs within ten random integrations [14]. Recently, by combining AAV technology with zinc finger nucleases, the efficiency of gene targeting increases up to 6% but most integration events still occur outside the target locus, most likely in naturally occurring DNA double-strand breaks [7,15-18].

Moreover, AAV-mediated gene targeting has been reported to be less dependent on the extent of homology between the vector and the genome target than other methods [14]. Notably, the presence of the ITRs flanking the gene targeting construct determines increased homologous recombination frequencies [19].

When AAV Rep proteins are expressed, the yeast *Saccharomyces cerevisiae* is able to replicate the ssDNA genome [20]. The proteins Rep68 and Rep40 are necessary for AAV replication and for site-specific integration [21,22]. Rep proteins interact with the Rep-binding element and the terminal resolution site sequences located within the ITRs, to create a nick that may increase the integration [23,24].

In the yeast *Saccharomyces cerevisiae*, gene targeting is very efficient; it occurs at a frequency of 2–3% and can be increased up to 25%, whereas random or non-homologous integration is reported to be less than 0.1% [25,26]. Yeast is an excellent genetic model for understanding the mechanisms and pathways involved in homologous and non-homologous recombination [27-30]. Moreover, yeast has been recently used as a system for studying the potential of a new genome editing approach for site-specific mutagenic and multiple allele replacement [31].

We decided to use the yeast *Saccharomyces cerevisiae* to evaluate whether the presence of ITRs in the integrating plasmid and the expression of AAV Rep proteins have an effect on gene targeting and random integration. The aim of this study was to assess whether the use of AAV sequences or expression of Rep proteins could be a feasible and valuable tool for increasing gene targeting or decreasing random integration.

Results and discussion

The presence of ITRs decreased the random integration of a gene targeting construct

AAV vectors are often used for gene targeting experiments in mammalian cells also in combination with zinc

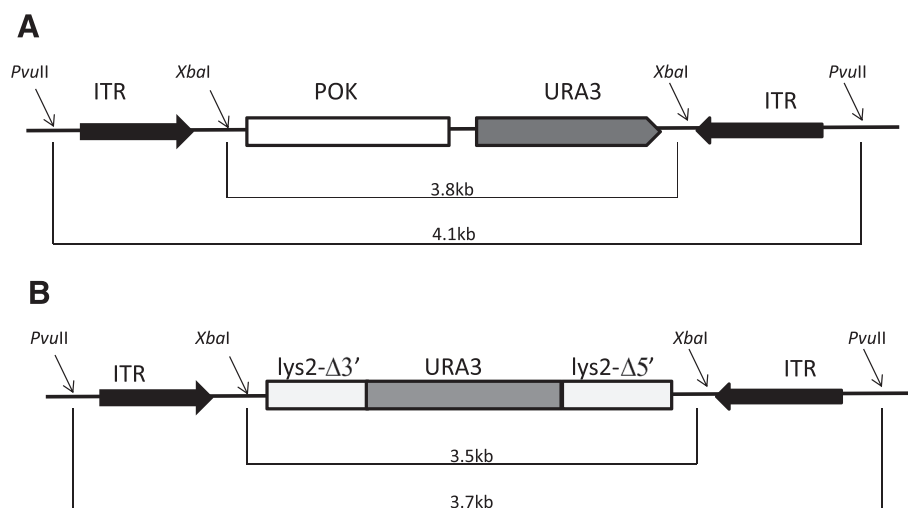


Figure 1 Schematic representations of plasmids carrying the recombinant AAV fragment (rAAV). **A)** pAAVpokURA. **B)** pAAVLUL. In both plasmids, restriction with *PvuII* gives rise to the rAAV fragment containing the ITRs; restriction with *XbaI* cuts out the ITRs and generates a fragment with no ITR at both ends.

Table 1 Effect of the AAV ITR sequence on gene targeting and random integration in yeast

Plasmid	Gene targeting	Random integration	GT/RI
ITR- <i>lys2URA3lys2</i> -ITR	31.0 ± 11.3 (7025)	0.62 ± 0.37 (487)	50.2
<i>lys2URA3lys2</i>	35.4 ± 10.4 (6960)	1.82 ± 0.41 (3420)*	19.4

Yeast was transformed with *PvuII*- or *XbaI*-restricted pAAVLUL as reported in Materials and Methods. Gene targeting and random integration events are reported as number of *URA3⁺lys2* colonies /10³ transformants per µg of plasmid DNA and *URA3⁺LYS2⁺* colonies /10³ transformants per µg of plasmid DNA, respectively. The number of transformants per µg of DNA was determined by transformation with 1 µg of episomal plasmid DNA. Results are the mean of four independent experiments ± standard deviation. The number of total colonies is indicated in parentheses. Statistical analysis was performed using Student's *t*-test. **p* = 0.0048 vs ITR-*lys2URA3lys2*-ITR.

finger technology [15,32,33]. Several studies indicate that AAV mediated gene targeting is affected by homologous recombination genes and that the AAV integration can be dependent on non-homologous end joining [34-37]; however, to our knowledge no comparative study has been performed to understand the role of ITRs and Rep proteins in gene targeting and random integration. Thus, to study the effect of ITRs on yeast gene targeting, we constructed a novel vector called pAAVLUL, containing the *LYS2* gene interrupted by *URA3* gene and flanked by the ITRs (Figure 1B). As shown in Figure 1B, the gene targeting fragment from the pAAVLUL was generated by two different restriction enzymes in order to keep the ITRs flanking both ends of the fragment, or not.

Overall, gene targeting was not affected by the presence of ITRs (Table 1); however, the random integration increased by almost threefold when ITRs were not present (Table 1). Thus, when ITRs are present, the GT/RI ratio increased fifty fold because the random integration decreased (Table 1). Southern blot analysis of genomic DNA of eleven *URA3⁺LYS2⁺* random integration clones derived from yeast transformed with ITRs-carrying fragments indicated that ten clones have a single *URA3* integration (Figure 2; clones 1, 3, 4, 5, 6, 7, 8, 9, 10, 11).

Although the size of the band is higher than the size of the AAV construct (3.7 kb) (Figure 1B), three clones out of ten have no ITRs as detected by the hybridization of the blot with ITR probe (Figure 2; clone 4, 5 and 9). Moreover, three clones have two or more copies of the construct, one detected with *URA* probe (Figure 2, clone 2) and two detected with ITR probe (Figure 2, clone 1 and 3). Clone 2 integrated two copies of the DNA fragment, but only one copy contained ITRs. We considered "rearrangements" those bands which were detected by only one probe (*URA3* or ITR) or those with a size smaller than rAAV (3.7 kb, Figure 1B). We can conclude that in six out of eleven clones (54%) some rearrangement occurred. In order to better understand whether these rearrangements are due to ITRs, we analyzed the genomic DNA of fourteen *URA3⁺LYS2⁺* random integration clones derived from the transformation with no ITR carrying-fragment. Figure 3 shows that nine out of fourteen clones (64%) contained a single copy of the fragment (Figure 3, clones 2, 3, 5, 6, 7, 8, 10, 12, 14) and there was no band lower than 3.5 kb (the size of the fragment without ITRs, Figure 1B). To make sure that ITRs are really not present in the genomic DNA, we hybridized the filter with the ITR probe. No bands were detected (data not shown). This result suggests that no rearrangement has occurred in absence of ITRs. Finally, we sequenced the junctions in order to see where the gene targeting construct was randomly integrated. By using two primers starting from the two portions of *LYS2* gene in the fragment, we were able to sequence four junctions; three junction sites were located on chromosome II right next to the *LYS2* locus and one junction was on chromosome XVI. The precise analysis of the sequence did not reveal any preferred junction site. However, it is possible that the homology between the fragment and the genome drives non-homologous integration. Our results clearly indicate that the presence of ITRs flanking the homologous sequence in the gene targeting

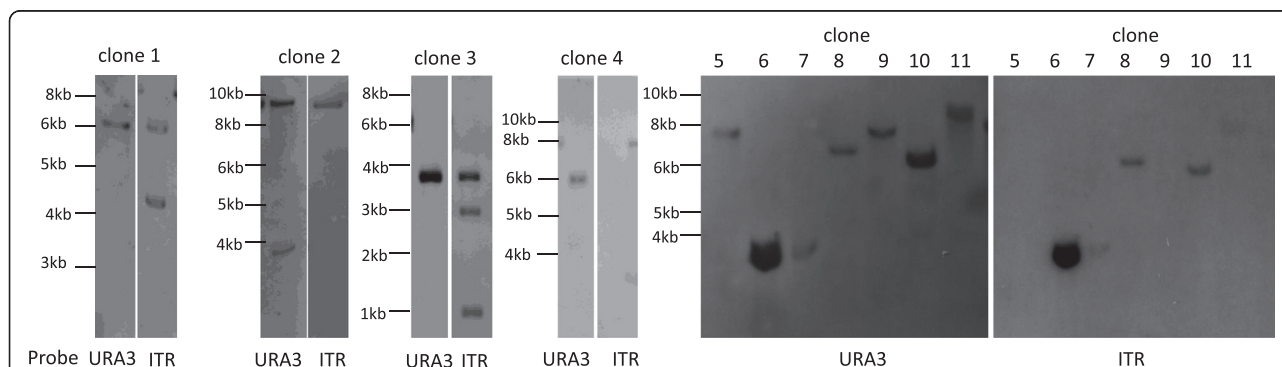
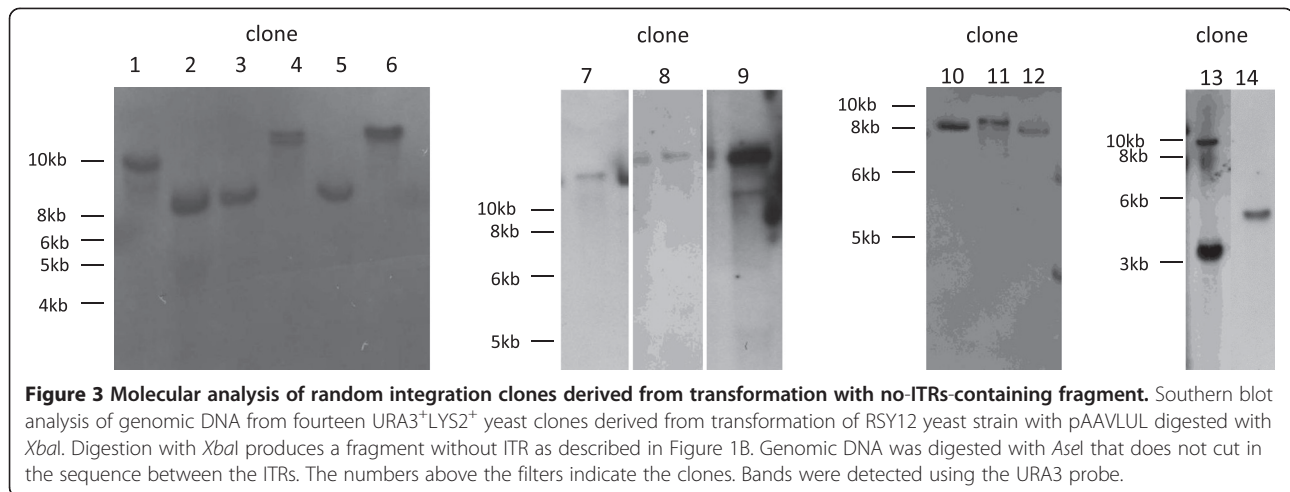


Figure 2 Molecular analysis of random integration clones derived from transformation with ITRs carrying fragment. Southern blot analysis of genomic DNA isolated from *URA3⁺LYS2⁺* yeast transformant clones. These clones were obtained by transforming RSY12 yeast strain with the ITRs-containing fragment obtained by the digestion of pAAVLUL vector with *PvuII*. We analyzed genomic DNA digested with *AseI* of eleven different clones. The numbers above the filters indicate the clones. Bands were detected using the *URA3* probe and the ITR probe as indicated.



fragment decreased random integration in yeast but determined a higher number of rearrangements.

The expression of Rep proteins increased AAV integration of a non-homologous fragment carrying ITRs

As Rep68 produces a site-specific ssDNA nick in the ITR, we thought that this ssDNA nick could affect both gene targeting and random integration. We used a yeast strain stably expressing all the Rep proteins to determine the effect on both gene targeting and random integration of the ITRs carrying fragment. The expression of Rep proteins is shown in the Western blot in Figure 4A.

When the integration fragment shared homology with the genomic target (*LYS2* chromosomal gene), both gene targeting and random integration were not affected by the expression of Rep proteins (Table 2). However, when there is no homology between the fragment and the genome locus (ITR-*pok*URA-ITR, Figure 1A), the Rep expression significantly increased random integration (see Table 2). Presumably, the ssDNA nick at the level of ITR may be repaired through non-homologous recombination, resulting in an increase in random integration of the vector. Southern blot analysis of the genomic DNA extracted from a total of twelve clones derived

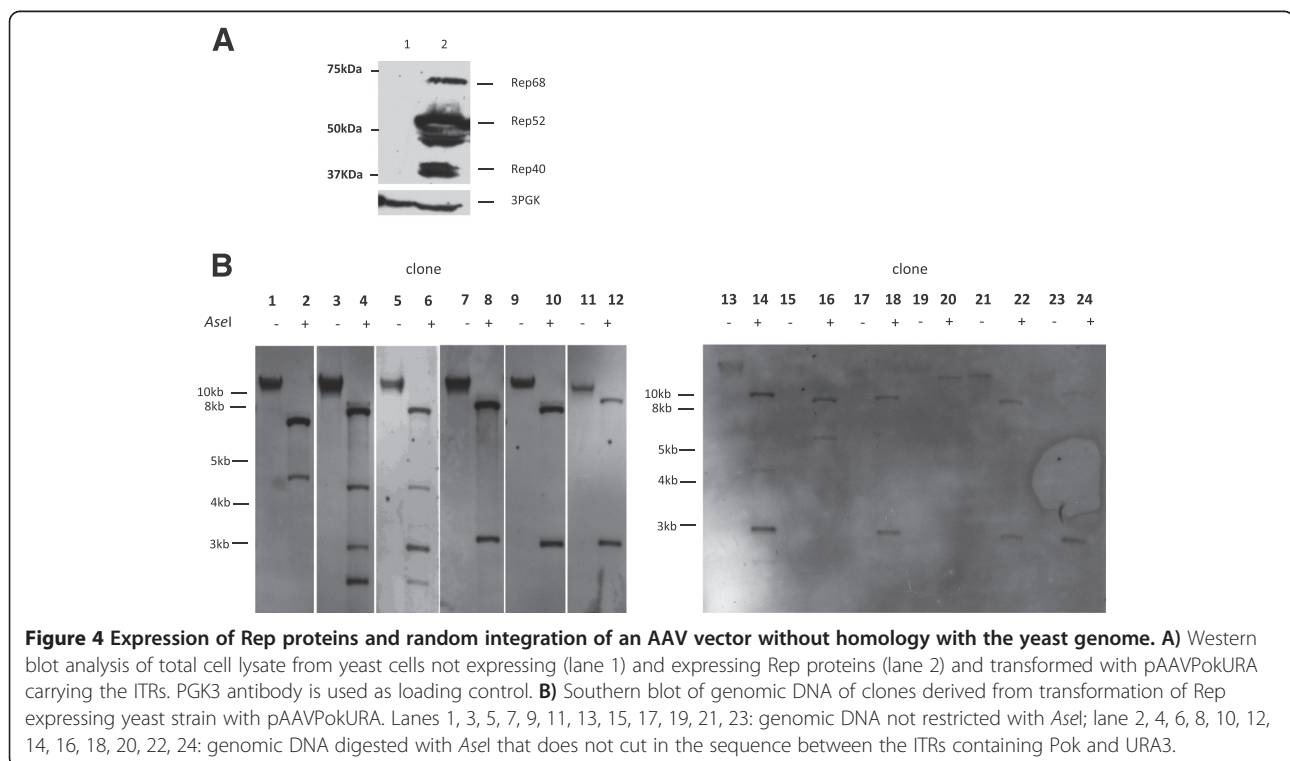


Table 2 Effect of the AAV Rep protein expression on gene targeting and random integration in yeast

Plasmid	Gene targeting	Random integration	GT/RI
ITR- <i>lys2URA3lys2</i> -ITR, No REP	31.8 ± 9.8	0.73 ± 0.39	43.5
ITR- <i>lys2URA3lys2</i> -ITR, REP	20.1 ± 5.9	0.88 ± 0.49	22.8
ITR- <i>pokURA3</i> -ITR, No REP	ND	0.12 ± 0.08	/
ITR- <i>pokURA3</i> -ITR, REP	ND	1.02 ± 0.56*	/

Yeast was transformed with *PvuII*-restricted pAAVLUL or pAAVpokURA as reported in Materials and Methods. In the case of experiments with pAAVLUL, gene targeting and random integration events are reported as number of *URA3⁺lys2* colonies /10³ transformants per µg of plasmid DNA and *URA3⁺LYS2⁺* colonies /10³ transformants per µg of plasmid DNA, respectively. Similarly, in the case of pAAVpokURA experiments, non-homologous integration events are reported as total *URA3⁺* colonies /10³ transformants per µg DNA. The number of transformants per µg of plasmid DNA was determined by transformation with 1 µg of episomal plasmid DNA. Results are the mean of four independent experiments ± standard deviation. The number of total colonies is indicated in the parentheses. Statistics was performed using Student's *t*-test. **p* = 0.0078 vs ITR-*pokURA3*-ITR, No REP. ND = not detectable.

from transformation of the yeast strain stably expressing Rep proteins was performed to check the number of stably integrated plasmid copies. It is interesting to note that eleven out of twelve clones contained at least two copies of the fragment (Figure 4B, lanes 2, 4, 6, 8 10, 12, 14, 16, 22, 24). This may be important for gene therapy application, where it is preferable to avoid vector random integration in order to prevent rearrangements.

Conclusions

Our results indicate that the ITRs and Rep proteins may affect AAV genomic random integration when no homology between the vector and the genome is present, and that rearrangements occur. Conversely, the presence of the AAV ITRs at both ends in a gene targeting construct sharing homology with a genomic locus could have an impact on the application of this strategy by significantly decreasing random integration.

Methods

Plasmids

The construction of the plasmid pAAVPokURA, which contains the URA3 marker and the 2.5 kb Pok stuffer sequence to increase the distance between the ITRs, has been previously reported [20] (Figure 1A). The plasmid pAAVLUL carrying the gene-targeting fragment between the two ITRs (Figure 1B), was constructed as follows: the 2.7 kb *EcoRI*-*Bam*HI fragment from pJZ102 [25] was first cloned into the pMCSsub; then, the entire fragment was cut off from pMCSsub with *Xba*I and inserted directly into the *Xba*I site of pSub201 containing the ITRs [20].

Yeast transformation and molecular analysis

The yeast strain RSY12 (*MATa leu2-3,112 his3-11,15 URA3::HIS3*) has a complete deletion of the *URA3* gene, which was replaced with the *HIS3* gene [38]. To evaluate

the effect of Rep proteins expression on gene targeting and random integration, we transformed the pAAV vectors in the RSY12 yeast strain containing the vector pG. Rep68 integrated into the genome [20].

Complete (YPAD) and synthetic complete (SC) medium were prepared according to standard procedures. Yeast was transformed with 3–5 µg of plasmid DNA using the standard lithium chloride method with single-stranded DNA as carrier [39]. The vectors pAAVLUL and pAAVpokURA were transformed, digested with either *Pvu*II or *Xba*I. Transformants were selected on SC-uracil (SC-URA). As the gene targeting events disrupt the chromosomal *LYS2* gene by inserting the URA3 marker, the URA3 transformants were replica-plated in SC-lysine-uracil (SC-LYS - URA) plates to score for the random integration and gene targeting. In parallel, the same yeast culture was transformed with an episomal plasmid to evaluate the efficiency of transformation per µg of plasmid DNA. The frequency of gene targeting and random integration, calculated by dividing the number of events by the transformation efficiency, was expressed as number of URA⁺lys2⁻/10³ and URA⁺LYS2⁺/10³ total transformants per µg of plasmid DNA, respectively. Data were statistically evaluated by Student's *t*-test with computer assistance. Single clones were grown in 5–10 ml of SC-URA and the genomic DNA isolated using the Master pure yeast DNA purification Kit (Epicentre Biotechnologies). The DNA was digested with *Ase*I, which does not cut in the rAAV fragment, electrophoresed, transferred to a nylon membrane (Roche), and hybridized with DIG-labelled -URA3 and ITR probes according to the standard protocol. DIG-labelled URA3 and ITR were obtained as previously described [20].

Western blot was performed with the monoclonal antibody 303.9 (PROGEN, Germany) and anti-3PGK (Invitrogen) as previously described [20].

To clone the junction sites, the genomic DNA from URA3⁺LYS2⁺ clones was digested with *Sph*I and ligated in the *Sph*I site of YEplac181 [40]. Then, the ligation mixture was transformed into competent *E.coli*. Single ampicillin-resistant colonies were grown in selection liquid medium, plasmid DNA extracted and sequenced with these primers: LYS2-UP 5' TCCACTGCCAAGTATAGAA 3' and LYS2-LOW 5'GTCATGTGGTAACACTGAA 3'.

Competing interests

The authors declare no competing interests.

Authors' contributions

AG and TC conceived of the study and participated in its design and coordination. TC carried out the experimental work and analyzed the data. AG had a major contribution in writing the manuscript. Both authors read and approved the final manuscript.

Acknowledgements

The authors are grateful to Robert Schiestl for the RSY12 yeast strain and the pJZ102 plasmid. The authors wish to thank Ana Backovic and Lidia Balestri

for technical assistance and helpful suggestions. They are grateful to Marcella Simili for reading and editing the final version of the manuscript.

Funding

This work was supported by Grant n. GGP09166 assigned by Telethon-Italy to AG. The funder had no role in study design, data collection, decision to publish or preparation of the manuscript.

Received: 16 October 2013 Accepted: 6 February 2014

Published: 13 February 2014

References

1. Sedivy JM, Dutriaux A: **Gene targeting and somatic cell genetics—a rebirth or a coming of age?** *Trends Genet* 1999, **15**:88–90.
2. Porteus MH, Baltimore D: **Chimeric nucleases stimulate gene targeting in human cells.** *Science* 2003, **300**:763.
3. Hockemeyer D, Wang H, Kiani S, Lai CS, Gao Q, Cassidy JP, Cost GJ, Zhang L, Santiago Y, Miller JC, et al: **Genetic engineering of human pluripotent cells using TALE nucleases.** *Nat Biotechnol* 2011, **29**:731–734.
4. Miller JC, Tan S, Qiao G, Barlow KA, Wang J, Xia DF, Meng X, Paschon DE, Leung E, Hinkley SJ, et al: **A TALE nuclease architecture for efficient genome editing.** *Nat Biotechnol* 2011, **29**:143–148.
5. Urnov FD, Rebar EJ, Holmes MC, Zhang HS, Gregory PD: **Genome editing with engineered zinc finger nucleases.** *Nat Rev Genet* 2010, **11**:636–646.
6. Pattanayak V, Ramirez CL, Joung JK, Liu DR: **Revealing off-target cleavage specificities of zinc-finger nucleases by in vitro selection.** *Nat Methods* 2011, **8**:765–770.
7. Gabriel R, Lombardo A, Arens A, Miller JC, Genovese P, Kaepffel C, Nowrouzi A, Bartholomae CC, Wang J, Friedman G, et al: **An unbiased genome-wide analysis of zinc-finger nuclease specificity.** *Nat Biotechnol* 2011, **29**:816–823.
8. van Nierop GP, de Vries AA, Holkers M, Vrijsen KR, Goncalves MA: **Stimulation of homology-directed gene targeting at an endogenous human locus by a nicking endonuclease.** *Nucleic Acids Res* 2009, **37**:5725–5736.
9. Di Primio C, Galli A, Cervelli T, Zoppe M, Rainaldi G: **Potential of gene targeting in human cells by expression of *Saccharomyces cerevisiae* Rad52.** *Nucleic Acids Res* 2005, **33**:4639–4648.
10. Kalvala A, Rainaldi G, Di Primio C, Liverani V, Falaschi A, Galli A: **Enhancement of gene targeting in human cells by intranuclear permeation of the *Saccharomyces cerevisiae* Rad52 protein.** *Nucleic Acids Res* 2010, **38**:e149.
11. Russell DW, Hirata RK: **Human gene targeting by viral vectors.** *Nat Genet* 1998, **18**:325–330.
12. Weitzman MD, Linden RM: **Adeno-associated virus biology.** *Methods Mol Biol* 2011, **807**:1–23.
13. Hendrie PC, Russell DW: **Gene targeting with viral vectors.** *Mol Ther* 2005, **12**:9–17.
14. Vasileva A, Jessberger R: **Precise hit: adeno-associated virus in gene targeting.** *Nat Rev Microbiol* 2005, **3**:837–847.
15. Ellis BL, Hirsch ML, Porter SN, Samulski RJ, Porteus MH: **Zinc-finger nuclease-mediated gene correction using single AAV vector transduction and enhancement by Food and Drug Administration-approved drugs.** *Gene Ther* 2013, **20**:35–42.
16. Handel EM, Gellhaus K, Khan K, Bednarski C, Cornu TI, Muller-Lerch F, Kotin RM, Heilbronn R, Cathomen T: **Versatile and efficient genome editing in human cells by combining zinc-finger nucleases with adeno-associated viral vectors.** *Hum Gene Ther* 2012, **23**:321–329.
17. Petek LM, Russell DW, Miller DG: **Frequent endonuclease cleavage at off-target locations in vivo.** *Mol Ther* 2010, **18**:983–986.
18. Gellhaus K, Cornu TI, Heilbronn R, Cathomen T: **Fate of recombinant adeno-associated viral vector genomes during DNA double-strand break-induced gene targeting in human cells.** *Hum Gene Ther* 2010, **21**:543–553.
19. Miller DG: **AAV-mediated gene targeting.** *Methods Mol Biol* 2011, **807**:301–315.
20. Cervelli T, Backovic A, Galli A: **Formation of AAV single stranded DNA genome from a circular plasmid in *Saccharomyces cerevisiae*.** *PLoS One* 2011, **6**:e23474.
21. Ward P, Urclay E, Kotin R, Safer B, Berns KI: **Adeno-associated virus DNA replication in vitro: activation by a maltose binding protein/Rep 68 fusion protein.** *J Virol* 1994, **68**:6029–6037.
22. Surosky RT, Urabe M, Godwin SG, McQuiston SA, Kurtzman GJ, Ozawa K, Natsoulis G: **Adeno-associated virus Rep proteins target DNA sequences to a unique locus in the human genome.** *J Virol* 1997, **71**:7951–7959.
23. Im DS, Muzyczka N: **The AAV origin binding protein Rep68 is an ATP-dependent site-specific endonuclease with DNA helicase activity.** *Cell* 1990, **61**:447–457.
24. Im DS, Muzyczka N: **Partial purification of adeno-associated virus Rep78, Rep52, and Rep40 and their biochemical characterization.** *J Virol* 1992, **66**:1119–1128.
25. Schiestl RH, Zhu J, Petes TD: **Effect of mutations in genes affecting homologous recombination on restriction enzyme-mediated and illegitimate recombination in *Saccharomyces cerevisiae*.** *Mol Cell Biol* 1994, **14**:4493–4500.
26. Galli A, Hafer K, Cervelli T, Schiestl RH: **The pol3-t hyperrecombination phenotype and DNA damage-induced recombination in *Saccharomyces cerevisiae* is RAD50 dependent.** *J Biomed Biotechnol* 2009, **2009**:312710.
27. Karpenshif Y, Bernstein KA: **From yeast to mammals: recent advances in genetic control of homologous recombination.** *DNA Repair (Amst)* 2012, **11**:781–788.
28. Krejci L, Altmannova V, Spirek M, Zhao X: **Homologous recombination and its regulation.** *Nucleic Acids Res* 2012, **40**:5795–5818.
29. Daley JM, Palmbo PL, Wu D, Wilson TE: **Nonhomologous end joining in yeast.** *Annu Rev Genet* 2005, **39**:431–451.
30. Chan CY, Schiestl RH: **Rad1, rad10 and rad52 mutations reduce the increase of microhomology length during radiation-induced microhomology-mediated illegitimate recombination in *Saccharomyces cerevisiae*.** *Radiat Res* 2009, **172**:141–151.
31. Dicarlo JE, Norville JE, Mali P, Rios X, Aach J, Church GM: **Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems.** *Nucleic Acids Res* 2013, **41**:4336–4343.
32. Khan IF, Hirata RK, Russell DW: **AAV-mediated gene targeting methods for human cells.** *Nat Protoc* 2011, **6**:482–501.
33. Luo Y, Kofod-Olsen E, Christensen R, Sorensen CB, Bolund L: **Targeted genome editing by recombinant adeno-associated virus (rAAV) vectors for generating genetically modified pigs.** *J Genet Genomics* 2012, **39**:269–274.
34. Vasileva A, Linden RM, Jessberger R: **Homologous recombination is required for AAV-mediated gene targeting.** *Nucleic Acids Res* 2006, **34**:3345–3360.
35. Paulk NK, Loza LM, Finegold MJ, Grompe M: **AAV-mediated gene targeting is significantly enhanced by transient inhibition of nonhomologous end joining or the proteasome in vivo.** *Hum Gene Ther* 2012, **23**:658–665.
36. Cataldi MP, McCarty DM: **Differential effects of DNA double-strand break repair pathways on single-strand and self-complementary adeno-associated virus vector genomes.** *J Virol* 2010, **84**:8673–8682.
37. Daya S, Cortez N, Berns KI: **Adeno-associated virus site-specific integration is mediated by proteins of the nonhomologous end-joining pathway.** *J Virol* 2009, **83**:11655–11664.
38. Schiestl RH, Petes TD: **Integration of DNA fragments by illegitimate recombination in *Saccharomyces cerevisiae*.** *Proc Natl Acad Sci USA* 1991, **88**:7585–7589.
39. Gietz RD, Schiestl RH: **High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method.** *Nat Protoc* 2007, **2**:31–34.
40. Gietz RD, Sugino A: **New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites.** *Gene* 1988, **74**:527–534.

doi:10.1186/1471-2199-15-5

Cite this article as: Galli and Cervelli: Inverted terminal repeats of adeno-associated virus decrease random integration of a gene targeting fragment in *Saccharomyces cerevisiae*. *BMC Molecular Biology* 2014 **15**:5.