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author's review

Yeast Transformation with Non-Replicative Plasmids: Different Genetic Alterations and the Choice of the Vector

Transformacija kvasca nereplikativnim plazmidima: različite genetičke promjene i odabir vektora

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Summary

The efficiency of yeast transformation with non-replicative plasmids, as well as the type of genetic alteration introduced, can be significantly influenced by different ***in vitro*** *modifications of the plasmid. The effects of conformation of the plasmid molecule (double- or single-stranded form), plasmid linearization with restriction enyzmes, DNA divergence between chromosomal and plasmidic sequences and UV-irradiation of the plasmid have been studied. These investigations can help us to better understand the mechanisms of genetic recombination but they might also be useful for improving the techniques of yeast strain construction. Introducing of foreign DNA fragments into the yeast genome by illegitimate integration of single-stranded plasmids or increasing of transformation efficiency by UV-irradiation of the plasmid are some of the possible applications of these investigations.*

Sažetak

Na uspješnost transformacije kvasca nereplikativnim plazmidima, kao i na tip uvedene genetičke promjene, može se značajno utjecati različitim ***in vitro*** *modifikacijama plazmida. Istraživan je utjecaj konformacije plazmidne molekule (dvolančani ili jednolančani oblik), linearizacije plazmida restrikcijskim enzimima, razlike u DNA sekvenciji između plazmida i kromosomalnog alela te ozračivanje plazmida UV-svjetlom. Ta istraživanja mogu pomoći u boljem razumijevanju procesa genetičke rekombinacije, a također mogu biti upotrijebljena u poboljšanju tehnika za konstrukciju sojeva kvasca. Unošenje stranih fragmenata DNA u genom kvasca ilegitimnom integracijom jednolančanih plazmida ili povećanje uspješnosti transformacije ozračivanjem plazmida s UV-svjetlom neke su od mogućih primjena ovih istraživanja.*

Introduction

Transformation of the yeast *Saccharomyces cerevisiae* with non-replicative plasmids yields transformants in which the plasmid DNA is either stably integrated into the genome or it donates genetic information to the chromosomal homologue by gene conversion (Fig. 1). Although in the latter case the plasmid molecule becomes lost during subsequent mitotic growth, such plasmids are sometimes also called integrative plasmids. The recombination process between plasmid molecule and chromosome, which always underlies transformation with non-replicative plasmids, occurs mainly between homologous DNA sequences *(1,2)*. However, the rare illegitimate integrations have also been observed *(3,4)*. Double-stranded (ds) plasmids linearized within region homologous to the yeast genome transform yeast at higher frequencies than their circular counterparts *(5;* Table 1). In this case plasmid integration is targeted into the chromosomal allele homologous to the cloned yeast gene in which double-strand break (DSB) was introduced. Furthermore, if a double-stranded gap (DSG) is made instead of DSB, the gapped molecule also transforms yeast cells with high efficiency and the gap beco-

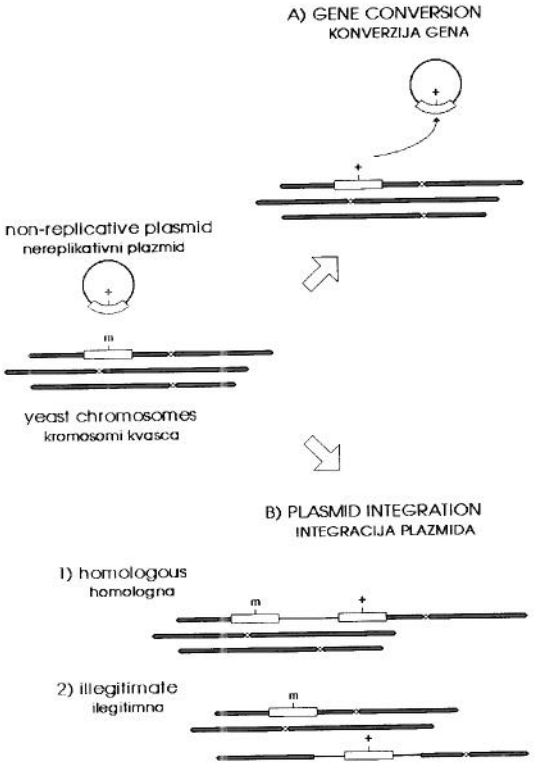

Fig. 1. Yeast transformation with non-replicative plasmids. Chromosomal allele is either replaced by the wild type copy of the gene (A) or the entire plasmid molecule becomes part of the yeast genome (B). Integration may occur within homologous (B1) or heterologous DNA-sequences (B2).
* + – wild tipe; m – mutation

Slika 1. Transformacija kvasca nereplikativnim plazmidima. Kromosomalni alel je ili zamijenjen divljim tipom gena (A) ili cijela plazmidna molekula postaje dijelom kvaščeva genoma (B). Integracija se može dogoditi unutar homolognih (B1) ili heterolognih DNA sekvencija (B2).
* + – divlji tip; m – mutacija

mes repaired during integration process *(1)*. The genetic information used in gap repair is from homologous region on the yeast chromosome.

These properties of yeast transformation made possible the development of several techniques suitable for creation of yeast strains differing in only one allele, such as gene replacement, gene disruption and the donation of the allele *(6-8)*. The general principle of these techniques is that the cloned yeast gene is first altered *in vitro*, and then used for transformation, in order to replace the wild-type chromosomal copy. During past few years we have developed several experimental systems based on transformation with non-replicative plasmids for studying the mechanisms of genetic recombination. We have shown that the transformation efficiency and the type of recombination event can be strongly affected by different modifications introduced into the plasmid molecule. Some of our results could be useful for improving the strategies of yeast strain construction. Here we present a short review of our results obtained in these investigations.

Single-stranded non-replicative plasmids and illegitimate integration

Single-stranded (ss) DNA is known to be involved in the recombination events in many diverse organisms. Circular non-replicative ss plasmids transform the yeast *S. cerevisiae* with manyfold greater efficiency than the corresponding ds plasmids *(2,9;* Table 1). We have found no further increase in transformation efficiency when targeted cuts are made within ss plasmid, unlike the effect observed with linearized ds plasmids *(10)*. However, genetic analysis of transformants obtained with these two conformations of ss plasmid indicated different distribution of transformation events. Linearized ss plasmids transformed yeast predominantly by illegitimate integration, even if the single-stranded break (SSB) was introduced within the DNA sequence with homology in the yeast genome *(11)*. Although some transformants obtained with circular ss plasmid were due to random plasmid integration into the yeast genome, most of them were produced by homologous recombination between ss plasmid and chromosomal allele. Another difference observed between linearized and circular ss plasmids was in their ability to convert the Ty transposon inserted in the *URA3* gene (*ura3-52* allele). Linearized ss plasmids converted this type of mutation with 5-fold higher efficiency than circular ss plasmids, while both conformations of ss plasmid converted the point mutation with the same efficiency.

All these findings were based on experiments in which plasmid DNA shared extended homology with the yeast genome. To investigate the ability of non-replicative plasmids (in ss or in ds form) to transform yeast cells via illegitimate recombination further we used the experimental system in which there was no homology between plasmid DNA and yeast chromosomes. In this case, both circular and linearized ss plasmids, transformed yeast cells with 100-fold higher efficiencies compared with the linearized ds plasmid *(11)*. Therefore, we suggest that ss non-replicative plasmids could be efficiently used for introducing foreign DNA fragments into the yeast genome, regardless of barrier imposed by homology requirement.

The influence of mismatches on yeast transformation with non-replicative plasmids

It has been previously shown that DNA-sequence diversity greater than 8 % almost abolishes recombinational potential between two DNA duplexes in yeast *(12)*. On the other hand, recombinational intermediates may involve DNA strands which are not identical. However, little is known about how low DNA-sequence polymorphism affects the recombination process. To get more insight into this problem, we have developed the experimental system in which non-replicative plasmids containing 0.1–0.7 % DNA-sequence diversity were used for yeast transformation *(13)*. These plasmids contained two yeast genes, so they could become integrated in either of the two corresponding homologous loci in the yeast genome. The smaller of the yeast genes (856 bp) present on the plasmid molecule contained 1, 2 or 6 nucleotides replaced, by site-directed mutagenesis. Trans-

Table 1. The effect of different modifications introduced in yeast non-replicative plasmids on transformation efficiency and the type of the recombination event
Tablica 1. Utjecaj različitih modifikacija u nereplikativnim plazmidima kvasca na uspješnost transformacije i tip rekombinacijskog događaja

Plasmid modification Modifikacija plazmida	Transformation efficiency* Uspješnost transformacije*		Recombination event Rekombinacijski događaj
None Bez modifikacije	ds	1	Homologous integration; gene conversion homologna integracija; konverzija gena
	ss	10–50	Homologous integration; gene conversion; rare illegitimate integrations homologna integracija; konverzija gena; rijetke ilegitimne integracije
Linearization within homology Linearizacija unutar homologije	ds	100–500	Targeted integration usmjerena integracija
	ss	10–50	Predominantly illegitimate integration pretežno ilegitimna integracija
Linearization outside of homology Linearizacija izvan homologije	ds	0.1	Illegitimate integration ilegitimna integracija
	ss	5–10	Illegitimate integration ilegitimna integracija
UV-irradiation (200–1000 J m^{-2}) UV-zračenje (200–1000 J m^{-2})	ds	10–50	Homologous integration; gene conversion homologna integracija; konverzija gena
	ss	1–10	Homologous integration; gene conversion increased homologna integracija; porast konverzije gena
DNA-sequence divergency (0.1–0.7 %) Razlika u DNA sekvenciji (0,1–0,7 %)	ds	0.3–0.5	Homologous integration; gene conversion homologna integracija; konverzija gena
	ss	10–50	Homologous integration; gene conversion; rare illegitimate integrations homologna integracija; konverzija gena; rijetke ilegitimne integracije

* approximative transformation efficiency relative to native double-stranded plasmids
* približna uspješnost transformacije s obzirom na nativne dvolančane plazmide

formants obtained with these plasmids, in either ds- or ss-form, were analyzed by Southern blotting to determine the exact site of plasmid integration. These analyses revealed that such low DNA-sequence polymorphism significantly affected the efficiency of integration, but only when the double-stranded vectors were used for transformation. For example, only one base pair substitution decreased the frequency of integration in homologous locus by almost 50 %. However, this effect was not proportional to the number of mismatches between two recombining duplexes. On the other hand, if mismatched base pair was already present on the plasmid molecule, an increase (up to 5-fold) in plasmid integration efficiency was observed *(14)*. This could be explained by a complex effect of DNA-sequence diversity on homologous recombination. Interestingly, transformation with ss plasmids was not sensitive to low levels of DNA-sequence polymorphism *(13)*. Therefore, ss plasmids could be used for integration of polymorphic DNA-sequences.

UV-irradiated non-replicative plasmids

UV-light induced lesions in DNA can be repaired by a process of genetic recombination *(15)*. Therefore, it was interesting to see whether UV-irradiation of plasmid DNA will stimulate recombinational transformation with non-replicative plasmids. Indeed, initial studies have shown that the integration was stimulated up to 50-fold if the plasmid DNA had been irradiated before transformation (*16*; Table 1). However, this effect was observed if both DNA strands received UV-treatment; irradiation of only one strand did not stimulate plasmid integration. It was concluded that the increased efficiency of transformation results from the recombinational repair of UV-induced lesions. The interplasmid recombination can also be stimulated by the UV-irradiation *(17)*. In another study we were able to follow the effect of UV-irradiation not only on plasmid integration, but also on transformation by gene conversion *(9,10)*. It was found that both transformation events are stimulated to the same extent, suggesting that recombination with irradiated and non-irradiated plasmid employs the same pathway. Interestingly, this was not the case when ss plasmids were used for transformation. In that case, the overall efficiency of transformation was diminished by the presence of UV-induced lesions, but the proportion of gene conversions was increased. Gene conversions represented almost 50 % of the transformation events when the plasmid molecule was irradiated with 1000 J m^{-2}, while non-irradiated plasmid yielded less than 10 % of gene conversions. Considering the universality of recombinational repair of UV-induced lesions we suggest the use of irradiated ds plasmids for transformation of organisms other than yeast in order to increase the efficiency of transformation. On the other hand, irradiated ss plasmids may be used for yeast transformation if gene conversion is the preferred genetic change in the genomic DNA.

References

1. T. L. Orr-Weaver, J. W. Szostak, R. J. Rothstein, *Proc. Natl. Acad. Sci. USA, 78 (1981) 6354.*
2. J. R. Simon, P. D. Moore, *Mol. Cell. Biol. 7* (1987) 2329.
3. R. H. Schiestl, T. D. Petes, *Proc. Natl. Acad. Sci. USA, 88* (1991) 7585.
4. R. H. Schiestl, M. Dominska, T. D. Petes, *Mol. Cell. Biol. 13* (1993) 2697.
5. T. L. Orr-Weaver, J. W. Szostak, *Proc. Natl. Acad. Sci. USA, 80* (1983) 4417.
6. S. Scherer, R. W. Davis, *Proc. Natl. Acad. Sci. USA, 76* (1979) 4951.
7. R. J. Rothstein, *Methods Enzymol. 101* (1983) 202.
8. C. Roitgrund, R. Steinlauf, M. Kupiec, *Mol. Gen. Genet. 237* (1992) 306.
9. M. Ninković, M. Alačević, F. Fabre, Z. Zgaga, *Mol. Gen. Genet. 243* (1994) 308.
10. Z. Zgaga, P. Koren, M. Ninković, K. Gjuračić, M. Alačević: Yeast non-replicative plasmids and strain construction. In *Proceedings of the 6th European Congress on Biotechnology*, L. Alberghina, L. Frontali, P. Sensi (Eds.), Elsevier Science B.V., Amsterdam (1994) pp. 327–330.
11. K. Gjuračić, Z. Zgaga, submitted for publication.
12. A. Adjiri, R. Chanet, C. Mezard, F. Fabre, *Yeast, 10* (1994) 309.
13. P. Koren, *MSc thesis*, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia (1994).
14. Z. Zgaga, R. Chanet, M. Radman, F. Fabre, *Curr. Genet. 19* (1991) 329.
15. E. C. Friedberg, *DNA repair*, W. H. Freeman Co., New York (1985).
16. Z. Zgaga, *PhD thesis*, Faculty of Food-Technology and Biotechnology, University of Zagreb, Croatia (1990).
17. Z. Zgaga, M. Alačević, *Prehrambeno-tehnol. biotehnol. rev. 29* (1991) 19.