ANADOLU, J. of AARI
10 (1) 2000, 87 - 96
MARA

NEW TRANSFORMATION SYSTEM: CHLOROPLAST TRANSFORMATION

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ABSTRACT: *Up to now a number of useful genes are transformed into higher plants via nuclear transformation. But nuclear transformation of crop plants has some potential problems. Those problems may be overcome by plastid transformation. Plastid transformation has several advantages over nuclear transformation.* ***Chlamydomonas reinhardtii****, green alga, is favourable organism for chloroplast transformation and used a model system for number of reasons. Chloroplast transformation techniques can be used to study on plastid genetic and molecular biology and transformation of foreign genes into chloroplast genome. Many useful genes have been transformed into chloroplast genome. On the other hand, it is clear that transformation of chloroplast genome is not easy and needs more care to manipulate of chloroplast genome.*

Keywords: Chlamydomonas reinhardtii*, plastid structure, chloroplast transformation.*

YENİ TRANSFORMASYON SİSTEMİ: CHLOROPLAST TRANSFORMASYONU

ÖZ: *Bugüne kadar yüksek bitkilere nükleus transformasyonu yoluyla birçok yararli gen transfer edilmiştir. Ancak bitki türlerinin nükleus transformasyonunda bazı potensiyel problemler vardır. Bu problemler plastid transformasyonu ile çözmek mümkün olabilir. Chloroplast transformasyonunun nükleus transformasyonuna göre birçok avantajları vardır.* ***Chlamydomas reinhardtii****, yeşil alg, chloroplast transformasyonu için değerli bir organizmadir ve birçok nedenlerden dolayı model sistem olarak kullanılmaktadır. Chloroplast transformasyon teknikleri plastid genetik ve moleküler biyolojisi üzerinde çalışmak ve chloroplast genomuna yabancı gen transferi yapabilmek için kullanılabilinir. Birçok yararlı gen chloroplast genomuna transfer edilmiştir. Diğer yandan chloroplast genomuna transformasyonunun kolay olmadığı ve manipulasyon için üzerinde daha çok durulmasına gereksinim olduğuda açıktır.*

Anahtar sözcükler: Chlamydomonas reinhardtii*, plastid yapısı, chloroplast transformasyonu***.

INTRODUCTION

It is possible to use of transgenic plants to study nuclear gene function and regulation and improve agronomically important crop plants. There are several alternative methods to produce of transgenic plants developed specifically for transformation of nuclear genome of higher plants (Svab *et al*., 1990).

DNA molecules can be introduced into plant cell via *Agrobacterium*-mediated DNA transfer (Fraley *et al*., 1985), electroporation (Fromn *et al*., 1986), calcium phosphate coprecipitation (Hain *et al*., 1985), polyethylene glycol treatment (PEG) (Paszkowski *et al*., 1984) or high - velocity microprojectiles (Klein *et al*., 1987) to manipulate and study on nuclear gene.

Introduction of foreign genes into nuclear genome of higher plant has become routine and a number of useful genes have been transformed into crop plants. Despite of tremendous application such transformed genes may get leaked into wild relatives due to cross pollination and pose big environmental problems (Mikkelson *et al*., 1996). Also the expression level is sometimes very low due to chromosomal position effects (Dean *et al*., 1988) and *trans* gene interacting (Jorgensen, 1990). All such problems may be overcome in plastid transformation.

Techniques for the transformation of plastid genomes have been developed. Introduction and stable integration of exogenous DNA have been reported in plastid genome of a unicellular alga, *Chlamydomonas reinhardhtii* (Boynton *et al*., 1988) and flowering plants (Svab *et al*., 1990).

PLASTID STRUCTURE

Plastids are a developmentally related class of organelles that carry out diverse cellular functions for plants and eukaryotic algae e.g., photosynthesis, the assimilation of nitrogen and sulphur, and the synthesis of carbohydrates, amino acids and fatty acids. During the evolution from prokaryotic photosynthetic endosymbionts, plastids have become tightly integrated within the cells they inhibit (Schmidt *et al*., 1981).

Proplastids of meristematic cells in flowering plants may differentiate into chloroplasts, amyloplasts or chromoplasts depending on the tissue type (Darnell *et al*., 1986; Mullet, 1988). Chloroplasts are the only type of plastid containing internal membrane (thylakoid). Thylakoid membranes contain chlorophyll, other pigments and enzymes that absorb light and generate ATP during photosynthesis (Palmer, 1991).

The biosynthesis of chloroplasts involves the contribution of two separate genetic systems. While most of their proteins are encoded by nuclear DNA and

imported into organelle form cytosol after they are synthesized on cytosolic ribosomes, some are encode by organelle DNA and synthesized on ribosomes within the organelle (Alberts *et al*., 1989).

Chloroplast DNAs of higher plants are circular molecules of 120 -160 kb and contain about 130 genes (Darnell *et al*., 1986; Palmer, 1991). Most of the genes that remain in chloroplast genomes encode components of the photosynthetic apparatus and the plastid genes expression system. This partitioning of the genes encoding plastid functions necessitates a co-ordination of the expression of the nuclear and plastid genomes (Rodermel *et al*., 1996).

ADVANTAGES OF CHLOROPLAST TRANSFORMATION

There are several advantages of chloroplast transformation over nuclear transformation.

Insertion of foreign gene into plastid genome may result in amplification of 50-100 copies of the gene per cell. In many particular species, all plastid types carry identical, multiple copies of the same genome (Palmer, 1991). There are 10-15 proplastids in meristamatic cells, each containing 50 genome copies. In a leaf cell, there may be as many as 100 chloroplast, each with approximately 100 copies of the plastid genome giving in total 10000 copies of the plastid genome per cell. It should be noted that there may be significant species specific deviation from these mean values, with a total number of genomes per leaf cell in the range of 1900-50000 (Maliga, 1993).

In plastid transformation there is no damage of the introduced gene getting leaked into wild relatives as plastid genes are inherited in (almost all) crop plant by the female parent only. Therefore relocation of nuclear genes to the plastid genome will confine to the transferred genes to the crop. Relocation of genes, such as those encoding herbicide resistance, to the plastids would prevent the transfer of herbicide resistance to the other species by cross - pollination (Maliga, 1993).

The codon usage of chloroplast genes which are close to prokaryotic genes are therefore a suitable place to express useful bacterial genes. Chloroplast genes are preceded by the -35 and -10 elements typical of prokaryotic promoters. (Stern *et al*., 1997) These genes transcribed by RNA polymerase containing plastid - encoded subunits homologous to the α, β and β subunits of *E. coli* RNA polymerase (Igloi and Kossel, 1992).

On the other hand, transformation of chloroplast genomes has some potential problems. Firstly, it may be more difficult for DNA to cross the plastid double

membrane than the nuclear membrane (Maliga, 1993). Secondly, chloroplast genomes are present in much higher copy number than nuclear genomes as mentioned above. For a transformed genome to replace all copies of the original genome, strong selection pressure must be applied (Chasan, 1992).

The transgenic plastid genomes are products of a multiple step process, involving DNA recombination, copy correction and sorting out of plastid DNA copies (Svab *et al*., 1990). Chloroplast genome can become somewhat unstable following transformation and that gene amplification represents a highly specialized phenomena that is not easily manipulated (Suzuki *et al*., 1997). Unintegrated plasmid DNA has also been detected in chloroplast transformants (Boynton *et al*., 1988). Similar problems are observed by Turkec (1999).

A MODEL FOR CHLOROPLAST ENGINEERING (*Chlamydomonas reinhardtii*)

First chloroplast transformation was reported by Boynton and co-workers in 1988 for unicellular alga, *Chlamydomonas reinhardtii*, using the biolistic method (Boynton and Gillham, 1993). *Chlamydomonas,* green alga which contains a single large, cup - shaped chloroplast with approximately 80 copies of its 196 - kb circular genome (Harris, 1989), has been favourable organism for number reasons.

This photosynthetic eukaryotic organism can be grown under controlled laboratory conditions in large amounts, and is thus suitable for biochemical analysis. *C. reinhardtii* cells, which exist either as mating type + or -, undergo a well-defined sexual cycle and are amenable to extensive genetic analysis. Cells can be propagated as well in the haploid as in diploid. Photosynthetic function in *C. reinhardtii* is dispensable provided a carbon source such as acetate is included in growth medium. It has therefore been relatively easy to isolate a large number of mutant s deficient in photosynthetic activity in *C. reinhardtii* as acetate - requiring strains (Harris, 1989). Nonphotosynthetic mutants resulting from deletions in the chloroplast *atp*B and *psb*A genes (Palmer *et al*., 1985) proved to be excellent transformation recipients. Chloroplast gene mutations conferring resistance to antibacterial antibiotics are easily selected (Harris *et al*., 1982). Efficient methods have been developed for nuclear and organelle transformation of *C. reinhardtii* (Boynton *et al*., 1988; Kindle, 1990).

Chloroplast genome of *Clamydomonas. reinhardtii* nearly the same set of photosynthetic and ribosomal protein genes as well as ribosomal RNA and tRNA genes that are found in the plastid genomes of higher plants. However, although chloroplast gene arrangement is highly conserved in most higher and lower plants.

This conservation does not extend to *Chlamydomonas* where chloroplast gene order differs markedly even between different species (Rochaix, 1995).

MANIPULATION OF CHLOROPLAST GENOME

Chloroplast transformation has three major requires. First, a method for DNA delivery through the double membrane of the plastid. Second, efficient selection for the transplastome. Third, integration of the heterologous DNA without interfering with the normal function of the plastid genome (Maliga, 1993).

Chloroplast transformation can be achieved by several ways. But to date only biolistic method (Svab *et al*., 1990) and polyethylene glycol (PEG) treatment (Golds *et al*., 1993) have yielded stable chloroplast transformation (Maliga, 1993).

Chloroplast transformation can be done with a particle gun in which cells are bombarded with DNA - coated tungsten particles in biolistic process.

Suitable selectable markers are requested in chloroplast transformation like nuclear transformation. Selection of the transformed copies of the genome can be accelerated by screening for spectinomycin resistance encoded by mutant 16S rRNA genes (Svab *et al*., 1990). A chimeric *aad*A gene encoding aminoglycoside-3-adenytransferase is also considered a suitable selectable marker in chloroplast transformation research (Svab and Maliga, 1993; Maliga, 1993).

During chloroplast transformation the transforming DNA integrates into the chloroplast genome (cp) via homologous recombination (Boynton *et al*., 1988). The transforming DNA can thus correct a chloroplast mutation by gene replacement, or a wild type gene can be replaced by e gene that has been mutated in vitro (Kindle *et al*., 1991). Furthermore, foreign genes can be incorporated into the cp genome by flanking them with cpDNA sequences to allow homologous recombination (Svab *et al*., 1990; Maliga, 1993).

USE OF THE CHLOROPLAST ENGINEERING

Chloroplast transformation techniques has been used to address plastid genetic and molecular biology such as characterization of promoter strength, trans - splicing , mRNA stability, photosynthetic function, to achieve targeted disruption of plastid genes, to examine the requirements for expression of foreign gene (Maliga, 1993). On the other hand, methods for chloroplast transformation have made it possible to identify sequence elements that regulate chloroplast gene expression in

vivo at the level of transcription (Klein,1992), transcript stability (Blowers, 1993), translation (Sakamoto *et al*., 1994) and photosynthetic complex assembly (Kuras, 1995).

Chloroplast can also be used for transferring of foreign genes. Many attempts are being made to transfer foreign genes to chloroplast. Some genes has already been transferred into chloroplast genome. Aminoglycoside adenine transferase (*aad*A), which confers resistance to aminoglcoside antibiotics, (Goldsmidt and Clermont, 1991; Svab and Maliga, 1993), bacterial genes encoding β-glucuronidase (*uidA*) (Sakamoto *et al*., 1992) and neomycin phosphotransferase (Career *et al*., 1993) are some of those genes expressed in chloroplast genome.

The chloroplast transformation system has many interesting features. Many researchers have taken advantage of the relative simplicity of the chloroplast genetic system compared to nuclear and prokaryotic systems to produce new findings and hypotheses which are not only restricted to the plant world but apply to other organisms as well (Sugita and Sugiura, 1996).

CONCLUSION

When we consider about chloroplast gene expression which is highly affected by environmental factors, it seems that more efforts and further research are needed in order to manipulate chloroplast genome as a model system so that chloroplast transformation system can be easily used for plant improvement researches.

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