A way to bypass cross-pollination

Friday, May 23, 2003
By Christof Fellmann, Checkbiotech

BASEL – Success in expressing tetanus toxin fragments in plant chloroplasts provides a potential route towards the development of safe, plant-based proteins.

Recently, Dr. Pal Maliga from the Waksman Institute, Rutgers University, NJ and his team succeeded in expressing a gene for tetanus toxin in tobacco plant chloroplasts. This tetanus toxin, also referred to as tetC, was used as a vaccine to successfully immunize mice against tetanus.

The revolutionary procedure developed by Dr. Maliga, to produce the tetanus vaccine, is very important because it allows for the generation of transgenic plants that virtually eliminate the concerns of cross-pollination. The reason is that genes enclosed in chloroplast DNA are not included in pollen, which prevents any new genetic material from being passed on to other plants.

In addition to this safety feature, there are two other important advantages of the chloroplast genome compared to the cell's main (nuclear) genome. One of them is that virtually any bacterial gene can be inserted into chloroplasts without being rejected. This opens a wide range of future application possibilities.

The second benefit is an increased level of protein (in this case tetC) production and therefore higher commercial potential. This arises because the inserted gene is more compatible with the cell machinery in chloroplasts than with the one in the nuclear genome.

To come upon the reason, early stages of evolution have to be considered. During that period some eukaryotic cells of plants and animals engulfed Bacteria (phagocytosis), their way of nutrition uptake. But instead of being destroyed the Bacteria were incorporated by the cell and became regular cell parts. This statement is referred to as endosymbiosis theory.

The chloroplasts, that originated as just explained, still possess a small genome that shows bacterial, and therefore prokaryotic, characteristics. The possibility to produce proteins through this bacterial machinery opens new gates for a wide range of applications.

Safer Gene Expression

Classically, genes for desired proteins are inserted into the nuclear genome. This technique has the disadvantage that genes can mix into genomes of other plants due to cross-pollination. Cross-pollination describes the exchange of pollen between plants of different varieties. Thus, progeny plants will contain genes from two different species which might give them a selective advantage.

In contrast, genes inserted into chloroplast genomes can not be passed on by pollens because they do not contain any chloroplasts. In fact, in most flowering plants chloroplasts are passed on maternally (by the egg cell) and are therefore excluded from any interaction with the main genome.

Nevertheless, in few cases there is a risk that inserted genes can move from chloroplasts to the nuclear genome. However, the majority of these genes will never produce any products.

Dr. Anil Day, professor at the University of Manchester notes, "This means that a tiny fraction of pollen (1/16,000) will contain transposed plastid genes. Most of these will be inactive."

“If pollen grains with these inactive transposed genes fertilize other plants the resulting hybrids will contain, but not express, the transposed genes. Unlike the original crop, which benefits from a functional trait gene in the plastid, the hybrids will not gain any advantage from defective nuclear-localised trait genes.”

This may be elucidated by an analogy. If you have a disc with a text written, for example on a Apple-computer, you will not be able to view it on a Apple-incompatible PC, nor will you be able to print it. Thus, you theoretically possess the information, but you can neither read it nor use it to profit from it.

Tetanus A Model Vaccine

While looking for possibilities to apply their knowledge, Dr. Maliga and his team found a way to produce a tetanus vaccine by expressing tetanus toxin fragments (tetC) in tobacco plant chloroplasts.

The team chose tetanus for several reasons. Dr. Gordon Dougan, from Imperial College, who provides the immunology expertise to the collaboration, explains the main points.

“Tetanus toxin is the prototype vaccine currently used throughout the world. The existing vaccine is an injectable vaccine that requires three doses for protection. Our aim would be to replace this with an edible/oral/nasal vaccine that protects with a single dose. The vaccine could also be used to boost immunity in adults.”

As a second reason he adds, “We also selected tetanus because it is a proven protective antigen with a simple correlate of immunity (antibodies to the toxin in the blood of vaccines).”

Thus with tetC a strong immune reaction is evoked, that leaves the body well protected from future infections of Clostridium tetani. Since the immune reaction is so strong, a successful immunization of mice can be easily monitored.

A Recipe To Transform Chloroplasts

Normally, researchers insert genes into the nuclear genome of cells. This process is referred to as transformation. Dr. Maliga’s approach is unique because he inserted the gene into chloroplast DNA. Since plastid is a general term for a group of cell parts comprising chloroplasts, their DNA is named plastid DNA (ptDNA).

The procedure of transforming chloroplasts, or generally plastids, is complicated because there are many plastid genomes in a cell compared to only one nuclear genome. Dr. Maliga explains how he successfully transformed all the plastid genomes:

“We change ptDNA then gradually eliminate the plastids with non-transformed copies by growing the cells in tissue culture, on antibiotic-containing medium. Expression of the tetC vaccine gene in the plastids of tissue culture cells is neutral - the transformed cells grow about as fast as the non-transformed cells.”

“Therefore, we link an antibiotic resistance marker to the vaccine gene. Expression of the antibiotic resistance gene makes the transformed plastids competitive in the tissue culture environment. When all ptDNA copies carry the vaccine gene, we can selectively remove the antibiotic resistance gene.”

Upon successful transformation, the cells are allowed to grow into adult tobacco plants, which will then produce the tetanus vaccine tetC in their leafs. TetC Expression In Tobacco Plant Leafs

In their experiments, Dr. Maliga and his team were able to produce two different tobacco plant lines. One plant line expressed tetC levels of 10% and was completely healthy. The second plant line expressed levels of 25% but had white leafs. This loss of color stems from a lack of chlorophyl and is referred to as chlorosis.

Commenting on these results, Dr. Maliga noted, “The plants that produce more than 25% tetC are growing slowly because production of the vaccine at this high level interferes with photosynthesis. However, plants that produce 10% tetC vaccine are perfectly normal. It makes sense to accept somewhat lower expression levels as a trade-off for having normal plants.”

“In the long run, we are looking for ways to separate production and plant growth. One solution is to selectively express vaccines in fruits, in the absence of vaccine expression in leaves, so that expression of the vaccine should not affect plant growth.”

Therefore, the next step will be to fine tune tetC expression in plastids in order to produce as much tetC as possible without effecting normal plant growth. But as Dr. Dougan, the team’s immunologist, says, “It will be a number of years before commercial use will become a reality. It will partly depend on whether we use tobacco, or some other plant as the vaccine antigen source, or how we deliver the antigen. Either way it will be 10 years before we reach the market.”