

DNA recombination for targeted plant breeding

Did evolution invent games of chance? During the development of sperm and egg cells, maternal and paternal genes are mixed at random, thus giving rise to new combinations of traits. What are the molecular mechanisms of this process known as DNA recombination? How can it be used to breed plants with higher yields? Prof. Dr. Holger Puchta and his team at the Karlsruhe Institute of Technology (KIT) are investigating the processes associated with the breaking up, unwinding, crossing over and rejoining of DNA strands in thale cress (*Arabidopsis thaliana*). In a strange twist, the researchers are also dealing with breast cancer genes.

Crossing over refers to a process that occurs during meiosis when the maternal and paternal chromosomes in the precursor cells of sperm and egg cells cross over and exchange extensive DNA fragments that carry genes. The exchange of genetic material between homologous chromosomes is key in the ability of species to adapt to changing environmental conditions such as increasing heat or the successive acidification of soil. Recombination produces new combinations of genes, which leads to the appearance of individuals that are better suited to dealing with a new situation. But what makes the DNA strands cross over? How do existing bonds in the DNA molecule break up, and how do new bonds develop? "All these questions also help us answer certain key issues involved in DNA repair," said Prof. Dr. Holger Puchta from the Institute of Botany II at the Karlsruhe Institute of Technology (KIT). "Answers to these questions might also support our efforts to exploit the mechanisms in medical and biotechnological applications."

The Holy Grail of breeders



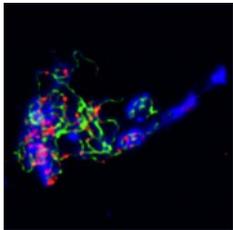
Thale cress blossom – *Arabidopsis thaliana* is a popular model organism in scientific research.
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As far as researchers can tell, DNA recombination takes place at random sites in the genome during the development of germ cells. Evolution "throws the dice" and more or less waits to see which of its experiments survives. However, Puchta and his team are interested in finding out whether the recombination mechanisms can also be used for specific applications. They believe that this would be the Holy Grail for breeders of agricultural crops. Crop breeders have always been interested in crossing suitable plants in order to make them more resistant to heat and high salt concentrations or to get them to produce higher yields. The researchers from Karlsruhe are therefore trying to gain an understanding of how the DNA of the cells of *Arabidopsis thaliana* is broken up and unwound, of the enzymes that catalyse the reconnection of DNA molecules and how the crossed DNA segments physically separate and become two separate

chromosomes once again. "We are initially looking for genes whose defective versions generate mutants in which specific DNA recombination steps no longer function properly," said Puchta.

The researchers also discovered a gene that they had not expected to find in *Arabidopsis*, namely a homologue of the BRCA2 gene, which has been found in mammals, including humans. Quite a large number of BRCA2 gene mutations have been found, several of which are associated with an increased risk of breast cancer. In addition, it has also been found that the product of the BRCA2 gene is normally involved in the repair of DNA damage such as random breaks that can spontaneously occur as a result of exposure to UV radiation and chemicals. "If the BRCA2 gene is defective or missing altogether, breaks in the DNA can no longer be repaired and mutations occur that can eventually lead to the degeneration of cells and to cancer," said Puchta. It was a revelation when homologues of breast cancer genes were found in plants in 2003. The discovery of these genes enabled researchers around the world to study in detail the molecular mechanisms of the effects of breast cancer genes. The silencing of these genes in animal models using genetic methods leads to the death of the embryos, whereas plant embryos survive.

Applying DNA scissors to selectively improve properties of species



Arabidopsis thaliana during meiosis. DNA is shown in blue; the red spots represent the location of the protein DMC1 that is involved in meiotic recombination; a structural protein that is found in the area where the paternal and maternal chromosomes pair.

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Puchta and his team silenced the BRCA2 gene and also manipulated the DNA repair system of *Arabidopsis thaliana* in other ways. This enabled them to show that the plant gene not only mediates the stability of the genome, but is also required for inheritance. The researchers also found other proteins that interact with breast cancer genes during the DNA repair process. At present, the researchers are working on finding out whether such interaction networks also play a role in germ cells, i.e. during the DNA recombination process that leads to offspring with new combinations of genes. To this end, the researchers are not just focusing on breast cancer genes that might lead to medical insights, but also on other candidates that play a role in the molecular processes associated with the recombination of DNA. They are focused, amongst other things, on RecQ helicases, enzymes that drive the unwinding of paired DNA. Mutations in human RecQ genes are implicated in heritable human diseases.

"In order to be able to carry out such experiments, we had to find ways to manipulate the DNA of our cells," said Puchta. Around twenty years ago, the biochemist was the first researcher in the world to use enzymes that enabled him to cut genetic information in the parent stock at specific sites and recombine, i.e. introduce new genes at these sites. Using these "DNA scissors", Puchta's group has been able to obtain detailed insights into the repair of DNA double strand breaks. The team has also been able to optimize a technique known as gene targeting. It is assumed that this technique will soon make it possible to use specific enzymes to specifically target defined genes in the genome of a broad range of different plants, modify the genes or replace these genes with genes from other plants.

According to Puchta, his research into genetic manipulation has huge potential for biotechnological applications. To this end, Holger Puchta was granted a European Research Council (ERC) Advanced Investigator Grant in 2011. This grant enables him to continue his promising work. "Using molecular scissors, we hope to control inheritance and transfer properties such as resistances to diseases, heavy metals and pests or genes that contribute to more rapid growth from wild to cultivated plants," said Puchta. "And best of all, the products we obtain have nothing to do with the classical and highly controversial genetically modified organisms. We do not use artificial genes, we use genes that already exist in natural organisms. We only recombine existing genes which is what happens in nature where sexual reproduction leads to new combinations of genes. The only difference is that we want to turn this natural arbitrary process into a controlled process of inheritance." In other words, targeted evolution that helps plant breeders to reach their goals faster than traditional methods would allow. These are still dreams of the future, but the grant awarded by the EU shows that Puchta's fundamental research has real potential.

Further information:

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