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‘Gene Scissors’ cause chaotic disturbance in plant genome

Chromothripsis demonstrated for the first time after CRISPR/Cas application in plants

Summary
Recent publications show that the application of gene scissors in plants is associated with a much higher risk of in-depth genomic disturbances than previously thought. Large areas of the genome can be involved.

Chromothripsis-like effects play a decisive role in this context. Chromothripsis refers to a phenomenon in which several hundred genetic changes can occur simultaneously in a 'catastrophic' event. Many sections of the genetic material can be swapped, twisted, recombined or even lost if this occurs. It was already known that these effects can be triggered by the use of CRISPR/Cas gene scissors in mammalian (and human) cells. Now, for the first time, chromothripsis-like effects have also been demonstrated in plants following the use of CRISPR/Cas gene scissors.

The exact mechanisms of chromothripsis are not yet fully understood. However, it is known that the simultaneous break of both strands of the genetic material can be a trigger for these chaotic effects. When both strands of DNA are cut, as is typically the case with the CRISPR/Cas gene scissors, the chromosomes lose contact with the ends that were separated in this process. If the repair of the break in the chromosomes fails, the separated end can be lost, or restructured and also incorporated elsewhere.

The use of gene scissors significantly increases the frequency of chromothripsis occurring. In addition, there is an increased probability that specific protected sites in the genome can be affected. The potential undesirable consequences include a wide range of risks such as disruption of plant health, altered interactions of the plants with the environment and undesirable changes in plant composition.

The results now available shed new light on the alleged precision of the gene scissors: New Genetic Engineering (New GE) can be used to target specific sites in the genome in order to knock out gene functions. However, the consequences of these 'cuts' into the genome are not predictable and cannot be controlled. Unintended genetic changes can affect large sections of chromosomes. Plants obtained from New GE procedures cannot therefore be considered 'safe', and risks must be thoroughly examined.

The application of gene scissors increases the likelihood of chromothripsis
If mutations occur, the cells attempt to repair the defect and, in many cases, can restore the original function. Since important gene functions are often particularly well protected from loss of function, mutations do not occur with the same frequency at every genomic site (Monroe et al., 2022).

New GE, however, differs from these natural processes: the use of the nuclease ('gene scissors') CRISPR/Cas usually causes both strands of the genetic material to be cut. If the cells try to restore the original gene function, the nuclease can recognize the repaired target region and cut there again, thus disrupting the repair processes. Especially in plants, the relevant genes are often present in multiple copies, and, therefore, the use of gene scissors typically results in several DNA double-strand breaks occurring in the genome and in a specific pattern (see, e.g., Sanchez-Leon 2018). The gene scissors also allow to alter genetic sites which are otherwise especially well protected from loss of function (Kawall, 2019).

It was already known that the use of gene scissors substantially increases the likelihood of chromothripsis occurring in mammalian (and human) cells (Ledford, 2020; Leibowitz et al., 2021; Amendola et al., 2022). In consequence, serious safety concerns have emerged regarding the use of CRISPR/Cas9 in clinical applications due to catastrophic DNA rearrangements, which are sometimes even addressed as ‘CRISPRthripsis’ (Amendola et al., 2022). Now, for the first time, these effects have been demonstrated after the application of CRISPR/Cas in plants. Before that, in plants, chromotripsis was already detected in the context of other genetic engineering processes (Chu & Agapito-Tenfen, 2022).

The mechanisms of chromothripsis
According to a recent publication (de Groot et al 2023), double-strand breaks are a trigger for chromothripsis. While not all the details of the process are fully understood, there is no doubt that use of the nuclease CRISPR/Cas, in particular, can significantly increase the probability of these effects occurring.

It is assumed that disrupted repair processes play a crucial role (de Groot et al., 2023): if cells do not immediately succeed in reconnecting the disconnected ends of the DNA, various processes can occur at those ends of the genome that were disconnected from the chromosome. These processes can affect large parts of the separated chromosome segments, and thus cause considerable chaos. Sections of the genetic material are incorporated in a twisted manner, duplicated, or may even be lost altogether. In some cases, the ends of the DNA of the dissected chromosomes can reconnect with each other despite these changes; in other cases, new connections are made to other sections of the chromosomes, which can lead to major changes in the structure of the genetic material (de Groot et al 2023). Large sections of the chromosomes may also be lost.

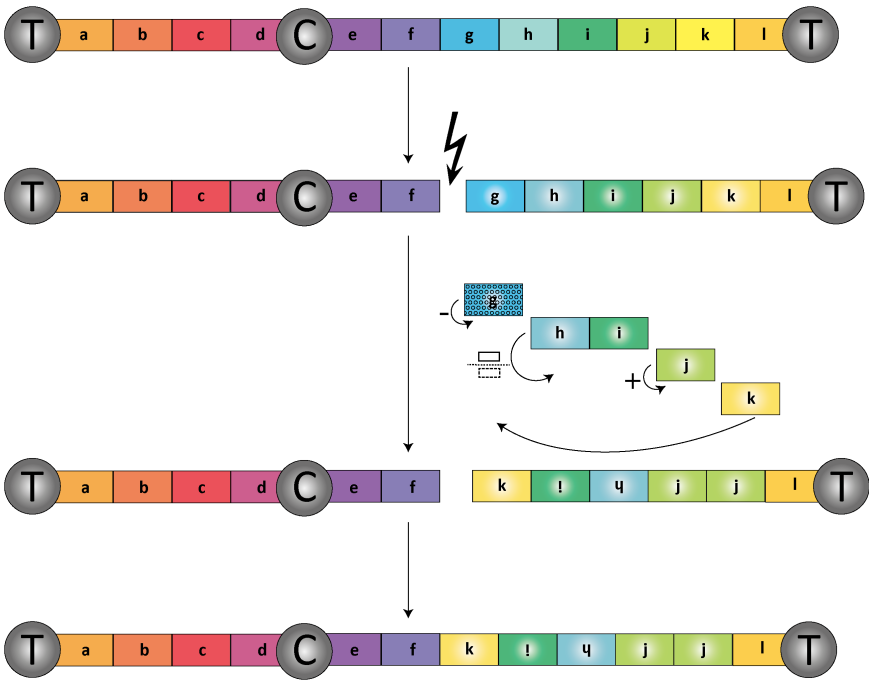

Figure: Examples for effects caused by chromothripsis: Various processes can occur at those ends of the genome that were disconnected from the chromosome. Sections of the genetic material are incorporated in a twisted manner, duplicated, or may even be lost altogether (adapted from de Groot et al., 2023).

Amongst other things, these processes can trigger various types of cancer in mammals (and humans) (Leibowitz et al., 2021, Amendola et al., 2022). In plants, the activity of genes can be altered, metabolic processes and growth can be disturbed or new constituents produced in the plants. This can also have negative consequences for health and the environment, for plant resistance to pathogens or climate stress. The risks cannot generally be assessed in advance, they must be investigated in each individual case.

First observations in plants

A recent study published (Samach et al., 2023) describes experiments in tomatoes. In order to identify chromothripsis after the use of CRISPR/Cas, the scientists first inserted additional genes that produce an easily recognisable colour.

The gene scissors were used in these plants to dissect the DNA on which the gene with the artificial marker was inserted from the rest of the chromosomes. The scientists would have normally expected the cells to repair this cut so that only this site was altered. The gene segments before and after it should have remained unchanged.

In some of the plants, however, the concentration of the color subsequently changed, indicating that the use of the gene scissors by no means only affected the target region: the marker was either lost or, in a few cases, became an even more intense color. The genetic material of these plants was subsequently examined more closely. This revealed a wide range of unintended consequences:

- In a few cases, the artificial gene marker was now found on both strands of the DNA, i. e. after the DNA had been broken, the affected gene segment had doubled (the trait became homozygous by somatic crossover). The resulting tomatoes showed an intense change in the colour of their flowers and fruits.

- In several cases, the colorant was lost because the particular chromosomal segment was affected by chromothripsis. In these cases, the loss of the entire chromosome or else major rearrangements of DNA, loss of major parts of the genetic material and new combinations of gene segments were observed.

The scientists (Samach et al., 2023) conclude that the use of gene scissors was a 'catastrophic event' that had triggered the process of chromothripsis. Until now, this effect in plants was unknown in connection with the gene scissors, probably because no one had conducted similar studies before. In fact, the effects of chromothripsis are not always easy to detect: if a targeted gene function is lost after the use of the gene scissors, this may be caused by an intended cut in the genome or by chromothripsin-like changes, thus involving much larger parts of the genome.

Although the peer review of the Samach et al. (2023) study is not yet completed, the results are plausible and consistent with other studies on the trigger mechanisms and effects of chromothripsis. Therefore, the experimental results should be duly considered in the further discussion on the regulation of the use of New GE in plants.

Relevance for the regulation of New GE plants

In the case of mammalian (and human) cells, the effects of ‘CRISPRthrispis’ triggered by the use of the gene scissors have been intensively researched for several years as they can, amongst other things, lead to developmental disorders and cancer (see Leibowitz et al., 2021, Amendola et al., 2022).

In contrast, this has only now been detected in plants in connection with the use of the CRISPR/Cas gene scissors (Samach et al., 2023). It was found that CRISPR/Cas applications can result in significant changes in the metabolism of plants, which can also have negative effects on health and the environment as well as jeopardize the future of plant breeding.

The results highlight an increased need for regulation: while breaks in the DNA can also be triggered by other factors, such as high doses of radioactive irradiation, it is doubtful whether these changes occur at the same sites with the same frequency, thus causing similar effects.

Generally, it can be assumed that the use of physical and chemical means to trigger untargeted genetic changes to increase genetic diversity will not cause events that would not be expected to occur naturally (see e. g. EFSA 2021).

The situation is different with the New GE: it is well known that the genetic changes induced by biotechnological mutagens (such as the gene scissors) and the resulting patterns may differ significantly from those expected from 'random' processes (overview at Koller et al., 2023). Inevitably, these effects also influence the genomic site and frequency of chromothripsis occurrence. Moreover, it seems not unlikely that the interactions of gene scissors with repair processes in the cell may favour or enhance the occurrence and course of chromothripsis.

Several stakeholders assume that the use of gene scissors will lead to an acceleration in breeding as their use is precise. It is now becoming clear once again that these frequently claimed advantages of using gene scissors cannot be delivered in this way: CRISPR/Cas can lead to numerous, far-reaching and unexpected genetic changes, which must be examined in detail not only with regard to safety but also with regard to the consequences for further breeding.

So far, the EU Commission seems to assume that it would be sufficient to consider only the intended genetic changes in the risk assessment of plants derived from New GE (see, for example, Testbiotech 2023). However, in this case, the effects of chromothripsis would in many cases remain undetected. The consequences and long-term effects will depend on many factors, such as specific gene combinations and environmental influences. The changes could also accumulate undetected in breeding populations over time, as well as threaten future plant breeding and food security.

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