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FUNCTIONAL ANALYSIS OF CT-DNAS IN NATURALLY TRANSFORMED PLANTS, RECENT FINDINGS AND GENERAL CONSIDERATIONS

Several cases have been reported of naturally transformed plant species. These plants contain cellular T-DNAs (cT-DNAs) derived from ancient infections by *Agrobacterium*. We have determined the structure of 4 different cT-DNAs in *N. tomentosiformis*, the paternal ancestor of *N. tabacum*, and found several intact open reading frames. Among these, TB-*mas2'* and TA-*rolC* were tested for activity. TB-*mas2'* encodes desoxyfructosyl-glutamine (DFG) synthesis. Some *N. tabacum* cultivars show very high TB-*mas2'* expression and produce DFG in their roots. The TA-*rolC* gene is biologically active and when expressed under strong constitutive promoter control, generates growth changes in *N. tabacum*. Based on these first data on the structure and function of cT-DNAs I present a theoretical model on the origin and evolution of naturally transformed plants, which may serve as a basis for further research in this field.

❦ **Kyewords:** cT-DNAs; natural GMOs; horizontal gene transfer; speciation.

INTRODUCTION

Pathogenic strains of *Agrobacterium* induce Crown galls and hairy roots on a large number of plant species. During the infection process, generally taking place wound sites, agrobacteria introduce one or several DNA fragments derived from tumor or root-inducing (Ti or Ri) plasmids. These DNAs are called T-DNAs (transferred DNAs, in case two T-DNAs are transferred these are called TA and TB or TL and TR) and contain several genes that will subsequently be expressed in the plant cells, leading to genetically transformed (transgenic) tissues. Many different *Agrobacterium* strains have been described and each contains its own type of T-DNA. T-DNAs are not always transferred intact, and many cases are known of partially duplicated tandem structures (either direct or indirect repeats, due to ligation of T-DNAs before insertion into the plant genome). Ti and Ri plasmids evolve most probably by horizontal gene transfer due to plasmid exchange between agrobacteria followed by recombination. Thus, the T-DNAs are mosaic structures and their evolution is difficult to reconstruct [1]. Many types of Ti and Ri plasmids exist and undoubtedly, many remain to be discovered. The T-DNA genes encode two major types of function. The first group encodes enzymes leading to the production of small conjugated molecules, called opines [2]. Opines are found in tumors and hairy roots, often at high concentrations, and many different types have been identified. Different substrates can be used for their production, all are important metabolites like amino acids, sugars and -keto acids. Opines are not found in normal plants. They are secreted from the transgenic tissues and taken up by the agrobacteria. Genes coding for opine uptake and opine degradation are located on the Ti/Ri plasmids and allow the bacterium to recover the original molecules. The second group of T-DNA genes enhances opine production by stimulating division and growth of the transformed cells. Among the genes of the Ti plasmids, the *iaaM* and *iaaH* genes code for auxin synthesis, the *ipt* gene for cytokinin synthesis, together they lead to undifferentiated growth. In the case of Ri plasmids, the *rolA*, *rolB* and *rolC* genes induce hairy roots by an as yet unknown mechanism [3, 4]. The *Agrobacterium* Ti/Ri-based transformation system provides an important selective advantage for agrobacteria, since other bacteria normally cannot use opines. The genetic manipulation of plants by pathogenic *Agrobacterium* strains aimed at providing important metabolites to the bacterium has been called genetic colonization [5].

Early in *Agrobacterium* research it was noted that several species of the *Nicotiana* genus contained T-DNA sequences [6, 7]. One of these, *N. glauca* (tree tobacco), was studied in more detail and found to contain an imperfect inverted repeat (called cellular T-DNA or cT-DNA) that resembled part of an *A. rhizogenes* T-DNA. This meant that somewhere in the evolutionary history of this species an *Agrobacterium* transformation event had occurred. Most likely, hairy roots were induced on a *N. glauca* ancestor, and spontaneously regenerated into fertile plants. The full structure of the *N. glauca* cT-DNA was only established much later. It consists of a partial inverted repeat, with an intact opine synthesis gene, the mikimopine synthase (*mis*) gene. Although this gene was shown to encode an active enzyme as demonstrated by expression in *E. coli*, no mikimopine could be found in *N. glauca* plants [8]. An extensive search in other plant species led to

the discovery of an imperfect direct cT-DNA repeat in *Linaria vulgaris* [9, 10]. Later, complete cT-DNAs were also described for *N. tabacum* (tobacco), *N. tomentosiformis* (the paternal ancestor of *N. tabacum*), *N. tomentosa* and *N. kawakamii*. Full sequencing of the *N. tomentosiformis* genome revealed the presence of no less than 4 different cT-DNAs (TA to TD). All are imperfect inverted repeats. These cT-DNAs were probably introduced by four successive transformations of an ancestor *Nicotiana* species by four different *A. rhizogenes* strains. The four T-DNAs partly resemble T-DNAs of known *A. rhizogenes* strains like 1724, but they also show several new features [11]. Interestingly, the TC cT-DNA was lost in *N. tabacum* by deletion, and the TA cT-DNA underwent a partial deletion in some *N. tabacum* cultivars, showing that cT-DNAs can undergo both small and large evolutionary changes. More recently, cT-DNAs were found in the agronomically important species *Ipomoea batatas* (sweet potato) [12]. It is likely that all cT-DNAs are derived from *A. rhizogenes* infections, with initial induction of hairy roots. Relatively little is known about the mechanism of induction and growth of hairy roots [13]. The *Nicotiana*, *Linaria* and *Ipomoea* groups thus contain natural GMOs, and other such plants will likely be discovered in the future.

One important question concerning these natural GMOs is whether the cT-DNAs are expressed, whether they might influence growth and possibly provide a selective advantage. In this paper I would like to discuss the evidence obtained so far, and provide a theoretical model for the origin and further evolution of natural GMOs.

Many open reading frames have been found on natural cT-DNAs. Some of these could be important in the growth or metabolism of natural GMOs. The possible role of cT-DNA genes in the origin and growth of plants has been studied by different groups. I first will discuss opine synthesis genes, then other cT-DNA genes.

ACTIVITY OF CT-DNA OPINE SYNTHESIS GENES

As mentioned above, the *N. glauca* cT-DNA *mis* gene encodes an active enzyme, but no mikimopine was found in *N. glauca* plants. In *N. tomentosiformis*, the TC cT-DNA contains an intact octopine synthase-like (*ocl*) gene. This gene was placed under strong, constitutitive promoter control (yielding 2 × 35S-*ocl*) and transiently expressed in *N. benthamiana*. Since *a priori* the opine structure could not be predicted, a negative staining method was used which can reveal the presence of opines when they are produced at high levels. A control gene, vitopine synthase (2 × 35S-*vis*), was tested in the same way and gave a positive response. Therefore, the *ocl* gene may not encode an active enzyme [11]. A third intact opine synthesis gene on the TB cT-DNA of *N. tomentosiformis* and *N. tabacum* is the mannopine synthase 2' (TB-*mas2'*) gene. This gene was already known

from T-DNAs of some *A. tumefaciens* and *A. rhizogenes* strains and encodes the synthesis of desoxyfructosylglutamine (DFG) in tumors and hairy roots. Notable differences exist between the *A. tumefaciens* and *A. rhizogenes mas2'* genes, the TB-*mas2'* gene belongs to the *A. rhizogenes* type. Using the same approach as for the *ocl* gene, it was found that TB-*mas2'* expression in *N. benthamiana* leads to DFG synthesis [11]. The next step was to look for DFG in *Nicotiana*. The expression levels of the TB-*mas2'* genes of *N. tomentosiformis* and most cultivars of *N. tabacum* were extremely low, but surprisingly, some tobacco cultivars showed very high levels of expression. The promoters of all TB-*mas2'* genes being identical, the differences in expression might be due to other genes (possibly transcription factor genes) present in high expression cultivars. Crosses between high and low expressors showed that the high expression phenotype behaved like a single mendelian factor. The expression pattern of the TB-*mas2'* gene was studied by the gene reporter approach (using a TB-*mas2'* promoter-GUS construct and a control A4-*mas2'* promoter-GUS construct expressed in *N. benthamiana*). These studies showed that the A4-*mas2'* and TB-*mas2'* genes are both preferentially expressed in root tips (in the case of A4-*mas2'* most likely in the tips of the hairy roots). This suggested that root tips of high expression tobacco cultivars might contain DFG, and this was indeed shown to be the case. DFG is an Amadori compound and is highly unstable because of rearrangements, but the DFG product in tobacco roots could unambiguously be identified by mass spectroscopy [14]. This was the first demonstration of opine production in a natural transgenic plant and showed that when looking for opines in natural GMOs, it might be helpful to first identify sites of high opine gene expression using reporter genes, and then search for the expected opines at these sites. Similar studies might be carried out with opine genes of other natural GMOs.

STUDY OF CT-DNA GENES OF THE PLAST TYPE

Among the other intact cT-DNA genes, only a few have been investigated so far. The first to be studied were *torf13* and *trolC* from the TA region of tobacco (these genes can also be called TA-*orf13* and TA-*rolC* but the older names will be used here to avoid confusion). Both belong to a large family of distantly related genes, the *plast* genes (for phenotypic plasticity) [15] and are found on the T-DNAs of *A. rhizogenes*. The *rolC* gene is directly involved in hairy root induction, whereas the *torf13* gene is not essential but can stimulate hairy root induction. The *torf13* was shown to have biological activity in root induction assays on tobacco leaf disks [16] and on carrot disks [17]. However, neither the transfer of *torf13* from tobacco cultivar Wisconsin to cultivar Samsun nn which has a partial TA deletion and lacks *torf13* genes, nor its stable expression

under $2 \times 35S$ promoter control in tobacco showed any morphological effects of this gene (Otten, unpublished results).

The *trolC* gene was tested by stable expression in tobacco under control of a dexamethasone-(DEX) inducible promoter in tobacco (dex-*trolC*). As a control, the A4-*rolC* gene from *A. rhizogenes* was used (dex-A4-*rolC*). The induction of both genes led to similar phenotypes: reduction of seedling growth, mosaic leaves with light and dark green areas, and accumulation of starch in leaves. Roots lost their root hairs and isolated roots became capable of growing on medium with low concentrations of sucrose. Isolated dex-*trolC* roots eventually stopped their growth whereas dex-A4-*rolC* roots continued to grow [18]. Thus *trolC* has biological activity.

SOME THEORETICAL CONSIDERATIONS CONCERNING NATURAL GMOS

The studies mentioned above show that at least some cT-DNA genes are biologically active. This raises the question about their possible function in the emergence and subsequent development of natural GMO plants. Although it is possible that natural GMOs are chance products of evolution and do not differ from their non-transgenic ancestors, this seems unlikely because the regenerants contain T-DNA genes like *rolA*, *rolB* and *rolC* known to influence plant development. The problem of cT-DNA gene function clearly needs to be separated in two parts: their role in the initial regenerants, and during the further evolution of such plants.

ORIGIN OF NATURAL GMOS

Natural GMOs result from the regeneration of tissues containing *A. rhizogenes*-type T-DNAs. Nothing is known about the regeneration of hairy roots to fertile plants under natural conditions, nor about the frequency of occurrence. Regeneration requires two conditions: the plant must be able to produce shoots from hairy roots, and the T-DNA structure must allow and/or favor such regeneration. Some species are well known for their capability to regenerate shoots from roots. Interestingly, *Linaria* is one of these. Tobacco can also regenerate easily: it was initially selected for tissue culture because of its excellent regeneration properties. Additional natural GMOs might be found by deep sequencing analysis of plants with a high regeneration capacity. It is likely that efficient shoot regeneration from hairy roots requires one or more special combinations of T-DNA genes in the hairy root tissues, each at specific expression levels. In this context it is interesting to note that the expression "hairy root" is extremely ill-defined in terms of root morphology and growth properties, and may cover different types of modified roots from highly abnormal to nearly normal. In order to test the hypothesis that some T-DNAs could lead to spontaneous shoot regeneration of hairy roots, one might choose as an example the TA region found in some *Nicotiana* species. One then needs to carry out the following experiments: 1. Identify a *Nicotiana* species close to the ancestor that received the T-DNA to be tested. In the case of the TA cT-DNA this could be *N. tomentosa*, which lacks the TA region. 2. Reconstruct the structure of the T-DNA that was introduced in the ancestor. The present TA sequences are partially mutated, degenerated and diverged (divergence between the repeats is 1.2 %). Reconstruction of the original TA T-DNA should be possible by comparison of the sequences of the repeated areas, eventually by sequence analysis of TAs from other *Nicotiana* species or accessions. 3. Infect the putative ancestor (here: *N. tomentosa*) with an *A. rhizogenes* strain carrying the reconstructed TA T-DNA. Since the TA region carries *rolA*, *rolB* and *rolC* genes, which are known to be essential and sufficient for hairy root induction, it can be expected that hairy roots will form. In the case of TA, these hairy roots will produce mikimopine. Each hairy root constitutes a clone, and many clones with different T-DNA structures and expression patterns might be generated at the infection site. It should be noted that we do not really know how much genetic variation can be expected for these clones, and whether initiation and growth of hairy roots from infected areas is subject to strong selection for certain T-DNA gene combinations or not. 4. Find conditions under which some of the hairy root clones form shoots. An extensive study on the phenotypes of these shoots, their cT-DNA structures and expression levels can provide us with a theory concerning the conditions (cT-DNA structure and expression) that will allow spontaneous regeneration of hairy roots and the probabilities of such conditions. 5. Study the fertility of a range of spontaneous regenerants and their capacity to generate progeny with the putative non-transformed ancestor. If some lines are fertile, but reproductively isolated from the ancestor, they are by definition new species, and the transformed plants might survive as natural GMOs. Reproductive isolation could occur by changes in flower structure or flowering time. It is also possible that the initial regenerants mainly reproduce by selfing (as in the case of tobacco), this would rapidly lead to clonal populations of transformed plants. The species within the Tomentosa group of the genus *Nicotiana* might potentially be explained by successive T-DNA transfer events. It is quite possible that horizontal gene transfer by *Agrobacterium* can create new species, which would enlarge the famous "origin of species by natural selection" [19] with an "origin of species by horizontal gene transfer". On the other hand, if the initial GMOs still formed fertile progeny with their ancestors, they would have disappeared from natural populations, unless the cT-DNA genes provided a selective advantage. After discussing the emergence of the initial natural GMO plants, their possible selection and maintenance over longer periods should now be considered.

MAINTENANCE OF NATURAL GMOS

It can be assumed that the genes of the T-DNAs that gave rise to natural GMOs were intact and led to classical hairy roots on susceptible plant species. However, present-day cT-DNAs carry various types of deleterious mutations. The TA region of *N. tomentosiformis* is strongly modified: only *torf13* and *trolC* are intact. The mutations could result from infection by an *A. rhizogenes* strain with an incomplete and mutated TA T-DNA, or from mutations that occurred in a hairy root and then allowed shoot formation. A third possibility is that the initial plants carried an intact TA cT-DNA and were relatively abnormal, with later mutations improving growth. If the original regenerant was reproductively isolated from the ancestor, no particular selective advantage was required for initial multiplication of such plants. However, subsequent cT-DNA mutations might progressively produce more efficient plants. Interestingly, the TA region is partially deleted in some tobacco cultivars, with loss of the two *torf13* gene copies but leaving the *trolC* genes intact. This could be fortuitous (gene erosion) or result from selection against some detrimental *torf13* effects.

ARE NATURAL GMOS A PART OF THE AGROBACTERIUM GENETIC COLONIZATION STRATEGY?

Tumors and hairy roots are opine factories and serve as cheap sources of sugars, amino acids and α-keto acids for the inciting *Agrobacterium* strains. Hairy root regenerants could continue to secrete opines from their roots which would lead to a large overall increase in opine production. If in addition, such isolated plants would be able to reproduce (either vegetatively as in the case of *Linaria* or sexually as in the case of *Nicotiana*) they would form regularly reproducing populations, thereby creating an even greater source of opines. Transgenic plants with active opine synthesis (most probably in the roots) would constitute a rich and stable source of opines for agrobacteria and might be considered as the ultimate step in genetic colonization. However, if opine production would diminish plant growth, these opine-producing plants would undergo selection for loss of opine synthesis and the benefit would only be temporary. On the other hand, a relatively low amount of opine production would not be detrimental to the plant but still of advantage for the bacterium. These considerations also raise the question of a possible advantage of opine production for the plant, for example by favoring the growth of plant-beneficial bacteria. For the moment, this seems to be a highly speculative possibility.

With regard to opine synthesis, one may expect two steps in the appearance of natural GMOs: 1. Regeneration of intact plants from hairy roots producing relatively large amounts of opines. 2. Appearance of mutants with less or no opine synthesis. In order to test the influence of opine synthesis on plant growth, opine synthesis genes of natural GMOs might be restored by CRISPR-Cas9 (in the case of the TA region, this would involve restoration of the two *mis* gene copies), followed by fitness comparisons with the non-restored plants. Any hypothesis proposing an initial selective advantage of natural opine-producing GMOs for *Agrobacterium* would also require a study of *Agrobacterium* populations in the rhizosphere of natural GMOs that secrete opines. So far, opine production has only been found in a few tobacco cultivars (like Russian Burley), these express the TB-*mas2'* gene to very high levels with concomitant production of DFG [14]. More studies are required on the regulation of the TB-*mas2'* gene, and the amounts of DFG that are synthesized and secreted, both under artificial and natural conditions. DFG can also be used by other bacteria and therefore does not constitute an ideal case [20]. It appears that the production of DFG in the high expression cultivars is a secondary phenomenon, since the TB-*mas2'* genes of the tobacco ancestor *N. tomentosiformis* and most other tobacco cultivars show only very low expression. High TB-*mas2'* expression might have been selected by tobacco growers and not occur in nature. Further studies will be required to determine whether DFG-producing tobacco plants occur in nature, whether they actively secrete DFG and whether this favors growth of agrobacteria or other microorganisms.

The possibility that agrobacteria use natural GMOs as opine sources could also imply a role for *plast* genes. In the case of *trolC*, it has been shown that this gene favors sucrose uptake [18]. As glucose and fructose are precursors for DFG, such *plast* genes might improve DFG production, especially if they are expressed in roots like the TB-*mas2'* gene. It is therefore important to learn more about the tissue-specific expression of the *trolC* gene, and its possible effect on DFG production. CRISPR-Cas9 may be used to remove the *trolC* genes or the TB-*mas2'* gene from the DFG-producing tobacco cultivars.

CONCLUSION

The various possible steps in the evolution of natural GMOs are summarized in Figure 1. Hopefully, some of the hypotheses evoked in this paper can be tested. The results will undoubtedly tell us more about the origin and the further evolution of the natural GMOs.

REFERENCES

1. Otten L, De Ruffray R. *Agrobacterium vitis* nopaline Ti plasmid pTiAB4: relationship to other Ti plasmids and T-DNA structure. *Mol Gen Genet*. 1994;245:493-505. doi: 10.1007/BF00302262.
2. Vladimirov IA, Matveeva TV, Lutova LA. Opine biosynthesis and catabolism genes of *Agrobacte-*

reconstruct putative ancestor from *Nicotiana* genomes

reconstruct T-DNA from:
1. cT-DNAs in different *Nicotiana* species and accessions
2. cT-DNA repeats

use reconstructed T-DNA to test HR induction on putative ancestor

produce HR regenerants (putative stage 4)

test consequences of opine production on growth at stage 4

compare plants of stage 4 with those of stage 7

test reproductive isolation of stage 7:

remove cT-DNA of stage 7 by CRISPR-Cas:

infection ancestral *Nicotiana* species: which one?

opine production in HR (stage 1)

opine production in HR root system in soil (stage 2)

opine production in roots of single HR plant (stage 3)

opine production in roots of independent HR plant (stage 4)

opine production in roots of HR plant population (stage 5)

Agrobacterium rhizogenes: which T-DNA structure?

Stage 1: induction HR on stems

Stage 2: hairy roots develop in soil

Stage 3: regeneration of shoot from a root clone with a particular cT-DNA structure and expression

Stage 4: fully independent HR plant

Stage 5: reproduction of HR plant: new species?

selective disadvantage over ancestor: maintenance by:
1. selfing or
2. reproductive isolation
3. vegetative propagation

selective advantage over ancestor: replacement

(selectively neutral)

Stage 6a: selection for cT-DNA mutations: better growth

Stage 6b: cT-DNA gene erosion

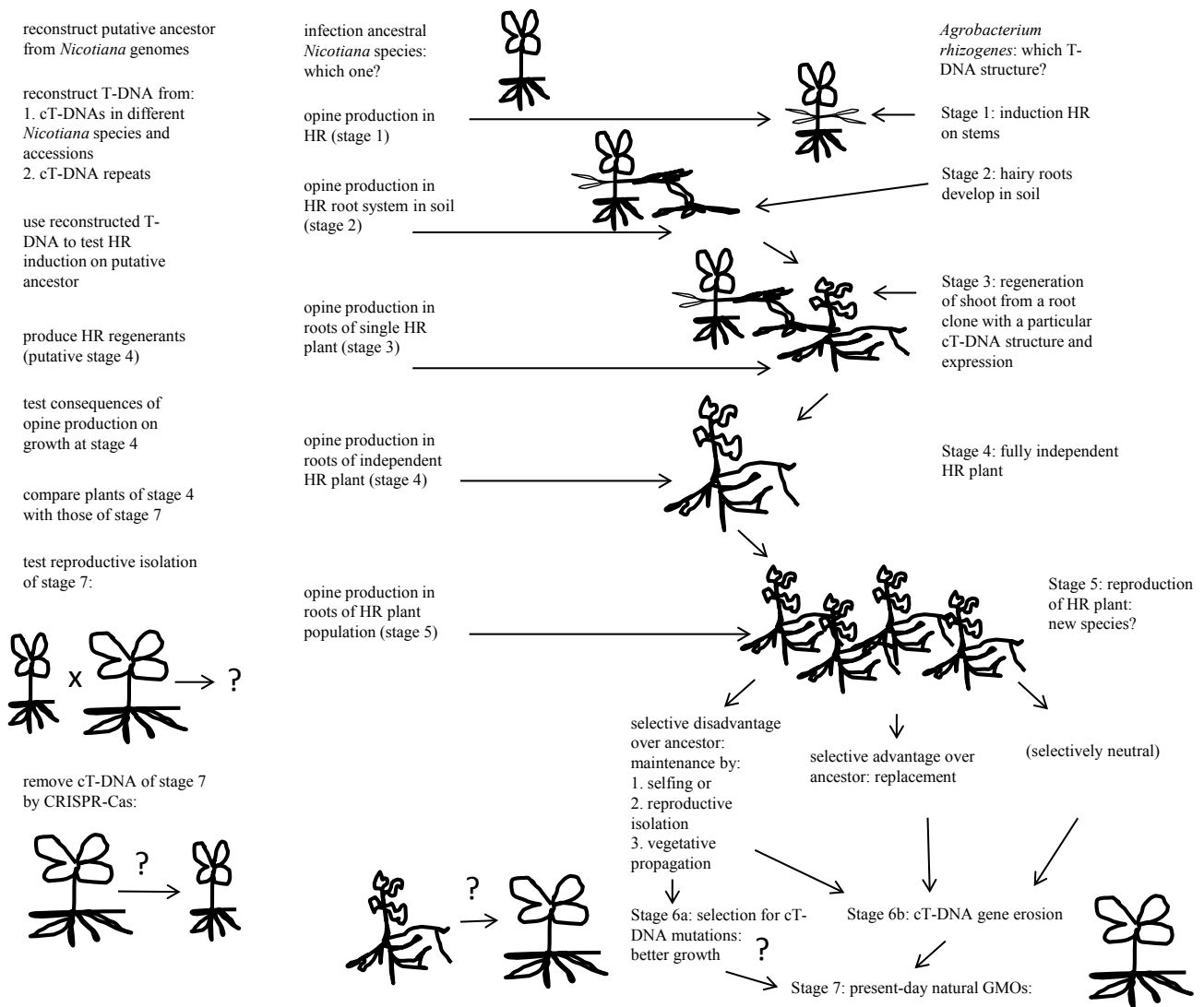Stage 7: present-day natural GMOs:

Fig. 1. Different hypothetical stages in the origin and further evolution of natural GMOs. On the left: possible ways to study the hypothetical events. The plant forms are symbolical and intend to show changes in morphology and physiology at the different steps. HR: hairy root

Рис. 1. Различные гипотетические этапы возникновения и дальнейшей эволюции природных ГМО. Слева: возможные пути для изучения этих гипотетических событий. Формы растений являются символическими и показывают изменения в морфологии и физиологии на различных этапах. HR: косматые корни

rium tumefaciens and *Agrobacterium rhizogenes*. *Russ J Genet*. 2015;51:121-129. doi: 10.1134/S1022795415020167.

3. Spena A, Schmülling T, Koncz C, Schell JS. Independent and synergistic activity of rol *A*, *B* and *C* loci in stimulating abnormal growth in plants. *EMBO J*. 1987;6:3891-3899.

4. Nilsson O, Olsson O. Getting to the root: the role of the *Agrobacterium rhizogenes* rol genes in the formation of hairy roots. *Physiol Plant*. 1997;100, 463-473. doi: 10.1111/j.1399-3054.1997.tb03050.x.

5. Schell J, Van Montagu M, De Beuckeleer M, et al. Interactions and DNA Transfer between *Agrobacterium tumefaciens*, the Ti-Plasmid and the Plant Host. *P Roy Soc Lond B Bio*. 1979;204:251-266. doi: 10.1098/rspb.1979.0026.

6. White F, Garfinkel D, Huffman GA, et al. Sequences homologous to *Agrobacterium rhizogenes* T-DNA in the genomes of uninfected plants. *Nature*. 1983;301:348-350. doi: 10.1038/301348a0.

7. Furner IJ, Huffman GA, Amasino RM, et al. An *Agrobacterium* transformation in the evolution of the genus *Nicotiana Nature*. doi: 10.1038/319422a0.

8. Suzuki K, Yamashita I, Tanaka N. Tobacco plants were transformed by *Agrobacterium rhizogenes* infection during their evolution. *Plant J*. 2002;32:775-787. doi: 10.1046/j.1365-313X.2002.01468.x.

9. Matveeva TV, Bogomaz DI, Pavlova OA, et al. Horizontal gene transfer from genus *Agrobacterium* to the plant *Linaria* in nature. *MPMI*. doi: 10.1094/MPMI-07-12-0169-R.

10. Matveeva TV, Lutova LA. Horizontal gene transfer from *Agrobacterium* to plants. *Front Plant Sci*. 2014;11(5):326. doi: 10.3389/fpls.2014.00326.

11. Chen K, Dorlhac de Borne F, Szegedi E, Otten L. Deep sequencing of the ancestral tobacco species *Nicotiana tomentosiformis* reveals multiple T-DNA inserts and a complex evolutionary history of natural transformation in the genus. *Nicotiana Plant J*. 2014;80:669-682. doi: 10.1111/tpj.12661.

12. Kyndt T, Quispe D, Zhai H, et al. The genome of cultivated sweet potato contains *Agrobacterium* T-DNAs with expressed genes: an example of a naturally transgenic food crop. *Proc Nat Acad Sci USA*. 2015;112:5844-5849. doi: 10.1073/pnas.1419685112.

13. Meyer A, Tempé J, Costantino P. Hairy root: a molecular overview. Functional analysis of *Agrobacterium rhizogenes* T-DNA genes. In Plant-Microbe Interactions, Vol. 5 (G. Stace, Keen N.T., eds). St. Paul, Minnesota: APS Press; 2000:93-139.

14. Chen K, Dorlhac de Borne F, Julio E, et al. Root-specific expression of opine genes and opine accumulation in some cultivars of the naturally occurring GMO *Nicotiana tabacum*. *Plant J*. 2016; *in press*.

15. Levesque H, Delepelaire P, Rouzé P, et al. Common evolutionary origin of the central portion of the Ri TL-DNA of *Agrobacterium rhizogenes* and the Ti T-DNAs of *Agrobacterium tumefaciens*. *Plant Mol Biol*. 1988;11:731-744. doi: 10.1007/BF00019514.

16. Aoki S, Syono K. Function of *Ngrol* genes in the evolution of *Nicotiana glauca* conservation of the function of *NgORF13* and *NgORF14* after ancient infection by an Agrobacterium rhizogenes-like ancestor. *Plant Cell Physiol*. 1999;40:222-230. doi: 10.1093/oxfordjournals.pcp.a029531.

17. Fründt C, Meyer AD, Ichikawa T, Meins F. A tobacco homologue of the Ri-plasmid *orf13* gene causes cell proliferation in carrot root disks. *Mol Gen Genet*. 1998;259:559-68. doi: 10.1007/s004380050849.

18. Mohajjel-Shoja H, Clément B, Perot J, et al. Biological activity of the *Agrobacterium rhizogenes*-derived *rolC* gene of *Nicotiana tabacum* and its functional relationship to other plast genes. *MPMI*. 2011;24:44-53. doi: 10.1094/MPMI-06-10-0139.

19. Darwin C. On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life, London, John Murray. 1859.

20. Baek CH, Farrand SK, Park DK, et al. Genes for utilization of deoxyfructosyl glutamine (DFG), an amadori compound, are widely dispersed in the family *Rhizobiaceae*. *FEMS Microbiol Ecol*. 2005;53:221-233. doi: 10.1016/j.femsec.2004.12.008.

ФУНКЦИОНАЛЬНЫЙ АНАЛИЗ КЛТ-ДНК В ПРИРОДНО-ТРАНСФОРМИРОВАННЫХ РАСТЕНИЯХ, ПОСЛЕДНИЕ РЕЗУЛЬТАТЫ И ОБЩИЕ СООБРАЖЕНИЯ

Л. Оттен

❀ **РЕЗЮМЕ:** Мы описали структуру четырех различных клТ-ДНК *N. tomentosiformis*, являющегося предковой формой *N. tabacum*, и обнаружили несколько интактных ORF. Среди них TB-*mas2*' и TA-*rolC* были тестированы на функциональность. TB-*mas2*' кодирует фермент синтеза дезоксифруктозилглутамина (DFG). Некоторые сорта *N. tabacum* характеризуются очень высоким уровнем экспрессии TB-*mas2*' и образуют DFG в корнях. TA-*rolC* биологически активен и будучи экспрессирован под контролем сильного конститутивного промотора вызывает изменения ростовых характеристик *N. tabacum*. Основываясь на данных о структуре и функции клТ-ДНК предложена теоретическая модель происхождения и эволюции природно-трансгенных растений, которая может быть использована при планировании последующих исследований.

❀ **КЛЮЧЕВЫЕ СЛОВА:** клТ-ДНК; природные ГМО; горизонтальный перенос генов; видообразование.

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