DNA Transfer to Plants by *Agrobacterium rhizogenes*: A Model for Genetic Communication Between Species and Biospheres

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David Tepfer

So what molecular biology has done you see, is to prove beyond any doubt but in a totally new way the complete independence of the genetic information from events occurring outside or even inside the cell, to prove by the very structure of the genetic code and the way it is transcribed that no information from outside of any kind can ever penetrate the inheritable genetic message.
Jacques Monod one of the founders of molecular biology, quoted in the Eighth day of Creation, by Horace Freeland Judson, Simon and Schuster 1979.

Abstract

Agrobacterium rhizogenes genetically transforms dicotyledonous plants, producing a transformed phenotype caused by the Ri TL-DNA (root-inducing, left hand, transferred DNA). Phenotypic changes include wrinkled leaves, reduced apical dominance, shortened internodes, changes in flowering, including a switch from biennialism to annualism, and altered secondary metabolite production, including increases in alkaloids. The transformed phenotype is correlated with a reduction in the accumulation of polyamines; it is mimicked using an inhibitor of polyamine synthesis. Roots transformed by *A. rhizogenes* grow in axenic culture, permitting the production of secondary metabolites in bioreactors, the modeling of the rhizosphere, and the propagation of arbuscular micorrhizal fungi for biofertilization.

A general view of parasexual DNA transfer postulates the exchange of genetic information among genetically distant plant genomes, with *A. rhizogenes* acting as an intermediary, thanks to its wide host spectrum for DNA transfer to plant,

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fungal, and animal cells and to exchange with other bacteria, including *Acinetobacter baylyi*, which uses homologous recombination to incorporate plant DNA into its genome. Marker exchange served to document DNA transfer from leaves and roots to *A. baylyi*. Transferred functions in this hypothetical system connecting phylogenetically distant genomes included genes encoding antibiotic resistance, nutritional mediators of plant/microorganism interactions (calystegins and betaines), and an elicitor of plant host defense responses (β-cryptogein), whose expression in tobacco resulted in increased resistance to *Phytophthora*. Thus, DNA encoding a trait of adaptive significance in a plant could be acquired by soil bacteria and eventually transferred into multiple plant species, thanks to the presence on the Ri TL-DNA of genes that increase developmental plasticity (organ formation) in the host plant, ensuring the sexual transmission of the foreign DNA. The image of genetic football is invoked to convey the multiple facets of this largely theoretical system of this parasexual DNA transfer.

The plausibility of a role for DNA transfer in the origin and future of our biosphere was tested by attaching unprotected DNA and seeds of *Arabidopsis thaliana* and tobacco to the outside of the International Space Station to simulate an interplanetary transfer of life. Seeds and fragments of DNA survived 18 months of exposure, indicating that DNA transfer could play a role in biosphere formation and evolution, particularly when protected from short wavelength UV by flavonoids in the seed coat.

Keywords

Agrobacterium rhizogenes • *Rhizobium* • *Phytophthora* • Arbuscular mycorrhizal fungi • Ri TL-DNA • Horizontal gene transfer • Rhizosphere • Polyamines • Panspermia • Evolution

Abbreviations

crypt	Gene encoding β-cryptogein
DFMO	DL-Difluoromethylornithine
DNA	Deoxyribonucleic acid
HGT	Horizontal gene transfer
nptII	Gene encoding kanamycin resistance
PCR	Polymerase chain reaction
Ri TL-DNA	Root-inducing left hand, transferred DNA
RNA	Ribonucleic acid
rolA,B…	Root locus A B… from the Ri TL-DNA

Contents

1 Introduction

The soil bacterium, *Agrobacterium rhizogenes*, naturally transfers DNA to dicotyledonous plant cells [1]. It uses the same DNA transfer mechanism as *Agrobacterium tumefaciens* [2], but in *A. rhizogenes* the transferred DNA (Ri T-DNA) is root inducing, while in *A. tumefaciens* the transferred DNA (Ti T-DNA) is tumor inducing (Fig. 1). (A right-hand, TR, DNA may also be transferred.) Tumor formation by *A. tumefaciens* results from the expression of genes carried by Ti T-DNA that encode the production of the plant hormones, auxin and cytokinin, in the transformed cells [3]. In contrast, the roots induced by *A. rhizogenes* regenerate shoots that carry Ri T-DNA into whole plants and their progeny [4]. These genetically transformed roots and shoots express the Ri T-DNA, and they are phenotypically altered in a similar fashion in different species [4]. Because the physiological basis for the transformed phenotype is still only partially understood, Ri T-DNA remains a source of information about how genotype leads to phenotype through conserved biochemical mechanisms. Some of the characteristics of the transformed phenotype have led to practical applications. See Sect. 9. This chapter describes the effects of Ri T-DNA on plant morphology, development, secondary metabolism, and plant/microorganism interactions. It will also consider *A. rhizogenes* in an ecological and evolutionary context, including in the coevolution of biospheres.

A comprehensive review would be too vast for this chapter, so in the name of brevity and simplicity no attempt is made to be exhaustive, and the reader is asked to search the literature for omissions of similar results that were published in parallel by other authors. Instead of writing a traditional review, I have taken the unusual step of recounting a personal history from an end-of-career vantage point, illustrating with

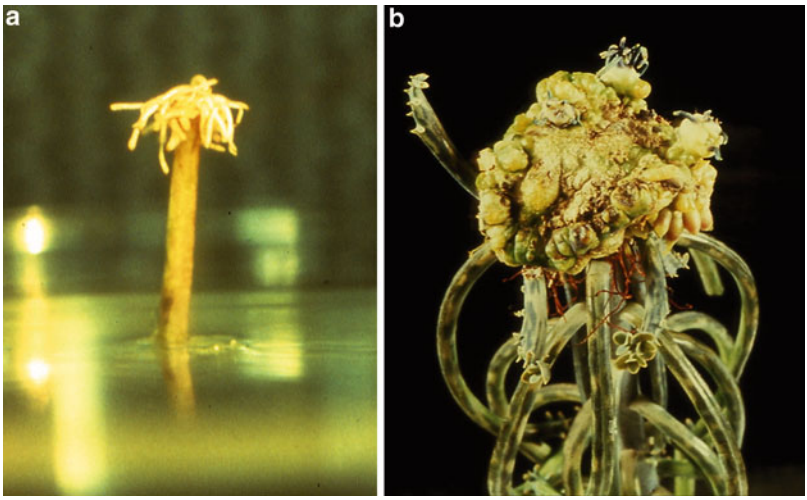

Fig. 1 Morphological responses at the site of inoculation. (**a**) root induction by *A. rhizogenes* on a morning glory (*Calystegia sepium*) stem segment; (**b**) tumor induction by *A. tumefaciens* on a decapitated kalanchoë plant (Photos D. Tepfer)

examples the ramifications that grew out of a simple initial observation and explaining how the human context molded the research.

1.1 Early Encounters

Seen in retrospect, lives and work are structured by early encounters. My father, Sanford (Sandy) Tepfer, was an evolutionary and developmental plant biologist at the University of Oregon (USA). My formative years were peopled by his colleagues, including Jacob (Jake) Strauss, who introduced me to plant molecular biology, and Howard (Howie) Bonnet, who taught me in vitro root culture, photobiology, and the scientific method. Family friends and neighbors included chemists, e.g., Virgil Boekelheide and Richard (Dick) Noyes; molecular biologists, e.g., Aron Novick, George Streisinger, and Sydney Bernhard; and microbial and plant ecologists, Richard (Dick) Castenholtz and Stanley (Stan) Cook; and botanists J. Strauss, H. Bonnet, and S. Tepfer. I remember Barbara McClintock and on another occasion the evolutionary biologist, E.B. Ford at our dining table – and meeting James Watson in a dark alley in Eugene, Oregon (coming out of a bar, arm in arm with Sydney Bernhard) when I was a teenager. There were frequent seminars in our living room and tea and cookies for students almost every evening. These encounters primed later interactions (see below) with botanists, chemists, microbiologists, molecular biologists, ecologists, and evolutionary biologists.

The idea of applying the just-emerging molecular techniques to plant biology came from my father's colleague, J. Strauss, who died young, shortly after I watched

him unpack a Sorvall centrifuge, a Cary spectrophotometer, and a fraction collector. These exotic treasures were attainable because the Russians orbited Sputnick around the Earth in 1957 (when I was 11 years old), causing an injection of Federal grant money into American science. My father's lab was quickly filled with graduate students and stacks of plastic Petri dishes. My presence there was tolerated, on the condition that I kept my hands in my pockets. The desire to get my hands on things in labs was thus born, and it has not waned nearly 60 years later.

In 1964, my family went to France for my father's first sabbatical leave, and I met Georges Morel and his graduate student, Arlette Ménagé (later Goldman), at the Institut National de la Recherche Agronomique (INRA) in Versailles. A. Goldman was working on the opines, which are exotic amino acid derivatives in axenic plant tissue cultures derived from tumors induced by *Agrobacterium tumefaciens* [5]. She was to become my principal collaborator in Versailles 15 years later.

Well before this first visit to INRA, Armin Braun had proposed that crown gall tumors could be caused, among other things, by genetic transformation [6], which induced the production of auxins and cytokinins in the transformed tissue [7] (Braun was influenced by his Rockefeller Institute colleagues, Oswald Avery et al., who first demonstrated genetic transformation in *Streptococcus pneumoniae* [8]). Specificity between opine synthesis in the transformed plant and opine catabolism in the bacteria was determined by the bacterial strain, and it was later put forward as evidence supporting DNA transfer in crown gall [9–11]. J. Tempé and Annik Petit later showed that opines induced the conjugation of the plasmid that encoded their catabolism [12], and opine synthesis genes were found in *Agrobacterium* T-DNAs. See reference [13]. Opine-like substances were also involved in the nitrogen-fixing relationship between *Rhizobium* and legumes [14].

My undergraduate research and Master's degree under H. Bonnet at the University of Oregon concerned the control by light of geotropism in morning glory roots grown in vitro [15]. The root culture and photobiology were to prove useful for producing axenic cultures of the roots induced by *A. rhizogenes* and in exposing plant seeds to space travel. Starting in the early 1970s, I was a graduate student at the University of California at Irvine (UCI), working under Donald (Don) Fosket, who encouraged me to follow-up on J. Strauss's ambition to do molecular biology in plants. Don had recently purchased a Sorvall and a spectrophotometer, and ultracentrifuges were available. My project stalled on the lack of methods for isolating RNA from plants but was saved by a timely publication [16]. I thus managed to do some pre-cloning molecular biology, concerning the control of protein synthesis by cytokinins [17, 18].

Howard Schneiderman was an important mentor at UCI. He had a Master's degree in botany but had devoted his Ph.D. and career to *Drosophila* developmental biology. He and Peter Bryant put Susan Germeraad, a fellow graduate student, on a project to genetically transform fruit flies by injecting DNA into their eggs. I was introduced through them to DNA transfer in its pre-embryonic stages, and it was several times the subject of departmental seminars, including one by Clarence Kado on *A. tumefaciens*, cytokinins, and crown gall. A few years later, H. Schneiderman launched genetic engineering in plants by convincing Monsanto Company that

agrochemicals should be replaced by transferred genes. I attended the first plant molecular biology conference in 1976 in Strasbourg, France, and after hearing Jozef (Jeff) Schell's seminar, I was convinced that crop plants would soon be fixing nitrogen. After UCI, I was a postdoc at the Molecular Biology Institute at UCLA, where I learned to manipulate DNA and to use in vitro translation systems. DNA cloning was new, and Gilbert and Maxim sequencing was being done with photocopied protocols, prior to publication. Biologists made their own electrophoresis equipment and enzymes. It was an exciting time to be a biologist.

A decisive move was precipitated by my father's proposal that we return to Paris for his third sabbatical. He suggested that I contact Jacques Tempé, who was working on crown gall, a field that was coming alive with the first Southern hybridization data showing bacterial DNA in axenic plant cell cultures induced by *A. tumefaciens* [19]. I had recently skied in Park City, Utah, with Ed Southern, inventor of the famous hybridization method, so everything seemed to point toward *Agrobacterium* research in France. I concocted a molecular biology project to work with J. Tempé in Versailles on the expression in plant cells of Ti T-DNA, and it was funded by NATO. I showed up, dripping with sweat, at INRA (Institut National de la Recherche Agronomique) in Versailles on a hot day in the fall of 1978, having rollerskated through the park behind the Louis XIV's palatial abode.

I was already a confirmed francophile and francophone (from my father's first sabbatical in 1964), but nothing prepared me for the shock of trying to do what I thought of as science at INRA. A French agricultural research station was very different from an American university. On the bright side, there were natural product chemists, including J. Tempé and A. Goldmann, excellent bacterial geneticists, including Jean Dénarié, Pierre Boistard, and Charles Rosenberg, working on *Rhizobium*, and a fine protein chemist, Jean-Claude Pernollet, working on a fungal elicitor. On the dark side was a vast array of often dysfunctional infrastructure and staff. (Even the electrical supply was unreliable.) Worse was the constant interpersonal conflict, often degenerating into sabotage. Aside from defective infrastructure and difficult human relations, there was no molecular biology (nor funding for it) and most of the necessary biochemical supplies had to be ordered from Sigma in the USA. I slowly became aware that in spite of the dysfunction and continuous infighting, novel collaborations with competent scientists could be forged in the unfamiliar subjects of plant breeding, pathology, soil science, nitrogen fixation, and natural product chemistry. I also slowly realized that the molecular project I had picked was not right for the environment at INRA.

A year of effort was wasted before arriving at the end of my fellowship and admitting defeat, but I was miraculously saved by a *deus ex machina* in the form of an unsolicited letter automatically extending my fellowship for another year. I was relieved to have a job but anxious to find a new project that was more in tune with local conditions, and I had only a year to obtain results. J. Tempé was on sabbatical leave in Australia. I needed to find a project that could be done quickly and that did not involve sophisticated molecular biology. For once, I felt free to explore, unencumbered by commitments to a pre-established research program.

2 *A. rhizogenes*, Root Cultures, the Transformed Phenotype

I saw Pierre Guyon washing fresh carrots in the lab sink. Later I saw him walk by with a tray of Petri dishes containing carrot disks on solid medium. A couple of weeks later I saw him carrying a tray of Petri dishes containing carrot disks covered with roots. That was my introduction to *A. rhizogenes*. He explained that he and J. Tempé were trying to find opines in the roots induced by *A. rhizogenes*. A paper had just appeared indicating that there were large plasmids in *A. rhizogenes* [20], and Tempé was hoping to use opines in the roots as evidence for DNA transfer.

I had used cultured morning glory roots to study the effects of light on geotropism under H. Bonnett [15], and I knew that excised carrot roots could be grown but with difficulty. I set up a series of carrot disks on agar + water medium, and I inoculated stem segments from morning glory plants growing behind the lab. Ten days later I had roots, which I excised, passed through multiple rinses in sterile water, and plated on White's root growth medium [21]. I directed the roots into the agar to keep their tips from sliding over the surface and carrying along contaminating *A. rhizogenes*, and I marked the apex of each root on the underside of the Petri dish. A career-changing surprise was waiting for me the next day. Many of the roots had elongated as much as a centimeter, which was completely unexpected based on my experience with morning glory. That evening I told my wife that I could not be leaving on the vacation in Corsica that we had planned with her parents.

The roots elongated as fast as a millimeter per hour and produced profuse laterals. Controls taken from germinated seeds grew slowly. I was quickly filling Petri dishes with roots, which I maintained as clones of each original root excised from the point of inoculation (Fig. 2). I was in root heaven. No bacteria grew when subcultured roots were crushed and plated on bacterial media. While passing through the medium, the roots had quickly outgrown the bacteria that had induced them. To my amusement, when the Petri dishes were full, the roots forced up the lids and grew across the shelf. The wild type transformed morning glory roots I had worked with in Oregon [22] had been slow growing in comparison, and they had rarely produced lateral branches. The only disappointment was that the morning glory roots did not regenerate shoots, while those in Oregon had produced shoots in response to light. I queried H. Bonnet, who surmised that I was working with *Calystegia sepium*, which does not regenerate. I inoculated *C. arvensis* stem segments, and a month later the resulting roots had produced formed shoots. I transferred the regenerants to soil in the green house, and to my surprise they had wrinkled leaves, and they were highly branched, like the roots they had come from. I used somatic embryogenesis to regenerate the carrot roots produced using *A. rhizogenes* A4, but the carrot roots induced by strain 8196 produced embryos directly from roots without hormonal treatment. Transformed tobacco roots regenerated without intervention, and inoculation of leaves sometimes produced plantlets directly [4]. All of the regenerants had wrinkled leaves and reduced stature and apical dominance (Fig. 3). The carrot plants had converted from biennial to annual flowering (see also Fig. 14). There was clearly

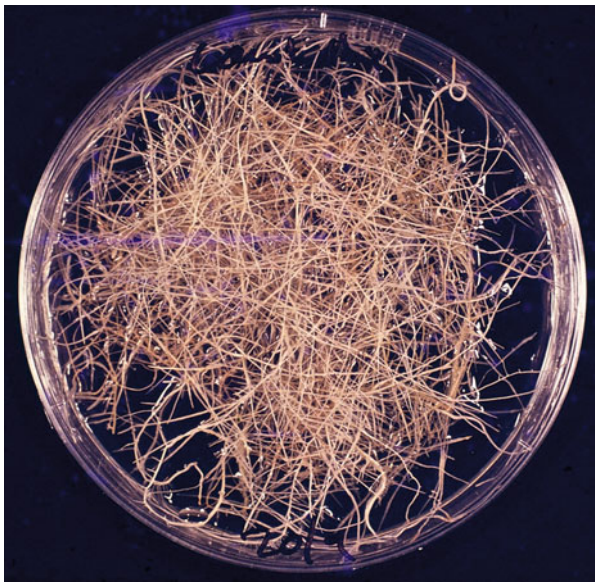

Fig. 2 Carrot root clone, genetically transformed by *A. rhizogenes* (Photo D. Tepfer)

Fig. 3 Changes in stature in tobacco, variety Mammoth, (regenerants from roots induced by *A. rhizogenes* strain A4), showing reduced apical dominance and wrinkled leaves. *Left* to *right*, wild type control, transformed plant of T phenotype, transformed plant of T' phenotype, transformed plant of an extreme T' phenotype. Ly-Yan Sun, the graduate student who made the plants, is on the far *left* (See also Fig. 5) (Photo D. Tepfer)

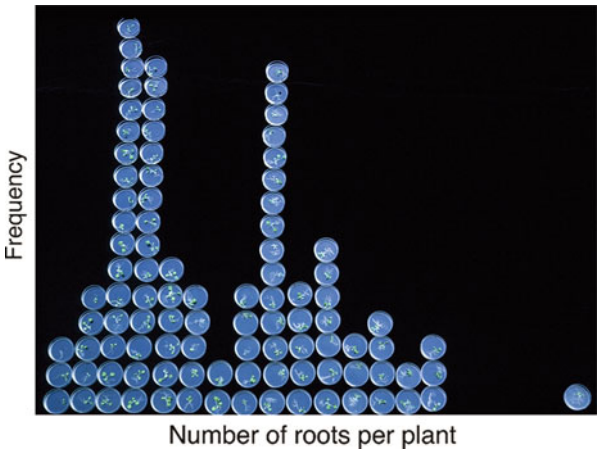

Fig. 4 Progeny of a transformed tobacco plant, variety Xanthi, resulting from crossing a wild type (female) with a primary transformant (male). Seeds were sewn in Petri dishes, which were sorted by root number per plant, starting with one root per plant on the *left*. The root number phenotype segregated as a 1:1 Mendelian dominant, with an outlier phenotype on the far *right* (Photo D. Tepfer)

a phenotype in both roots and regenerated plants that was similar in different plant species and similarly induced by the two bacterial strains I had used (A4 and 8196). This transformed phenotype included a variety of morphological changes and a surprising increase in regeneration capacity. It remained to show that roots and whole plants were genetically transformed.

J. Tempé returned from sabbatical leave in Australia to find his lab filled with roots. He had learned how to use high voltage paper electrophoresis to assay for two new opines, agropine and mannopine, whose synthesis is encoded by T-DNA and whose catabolism is encoded by the bacterial plasmid that carries the T-DNA. My *A. rhizogenes* A4 roots contained agropine in surprisingly high amounts and those induced by *A. rhizogenes* 8196 contained mannopine [23], providing evidence for genetic transformation. I went to Mary Dell Chilton's lab in Saint Louis (USA) to do Southern hybridizations with root and plant DNA that I had extracted in Versailles. I had to return to Versailles before the films were developed, and when they finally were, the results were positive but grossly over exposed.

Just as I had finished setting up to repeat the Southern blots in Versailles, J. Tempé expelled me from his lab to make way for a visit from M.D. Chilton to repeat the Southern blots with DNA from roots produced under my tutelage by a student in a neighboring lab. I set up my Southern hybridization experiments in another lab in Versailles, using DNA from roots and regenerated plants. All of the results came in within a few days of each other. There was T-DNA from *A. rhizogenes* in the roots that M.D. Chilton analyzed [24] and in all of the roots and plants that I had produced, including their progeny [4, 25–27]. The transformed genotype was inherited as a Mendelian dominant in tobacco (Fig. 4) [4, 25–28], and it co-segregated with the transformed phenotype in morning glory, carrot, and tobacco. In tobacco, it came in two general intensities, T and T', with the more intense, T' phenotype reverting to the T phenotype in lateral shoots (Fig. 5). The plants of T phenotype were homozygous for the T-DNA insertion and the T' plants were heterozygous [29]. See references [30–35]. In tobacco, the ability to grow under reduced gas exchange,

Fig. 5 Reversion of the T' phenotype to T phenotype in tobacco, variety Xanthi (Reprinted from Ref. [28]) (Photo D. Tepfer)

attributed to wild type Ri T-DNA [4], was later explained by a reduction in ethylene production [36].

In the meantime, a colleague in Versailles, Jean-Pierre Bourgin, had alerted me to a paper in German, describing earlier experiments in which tobacco roots induced by *A. rhizogenes* had been converted to callus, which had regenerated into plants, showing the altered morphology I had described [37]. Thus, transgenic plants had been produced using *A. rhizogenes* before mine. The authors' conclusion that they were genetically transformed was substantiated by sexual transmission of the phenotype. I cited their work in papers and seminars, and I invited the first author, Claudius Ackerman, to do Southerns and Northerns in Versailles, which proved that the descendants of the plants he had produced in 1973 [37] were genetically transformed. He had produced transgenic plants well before mine, but it turned out that nature had done it before all of us.

Clearly, no human intervention should be necessary for gene transfer from *A. rhizogenes* to morning glory, since root production at a wound site in the soil would produce a secondary root system, which would regenerate transformed plants that would pass the foreign genes on to their progeny. In keeping with this thought experiment, positive hybridization signals were reported with Ri plasmid probes in wild type *Nicotiana* [38] and *C. arvensis* plants [25]. Ancient Ri TL and TR-DNAs were recently described in domesticated sweet potato (*Ipomoea*) [39], a member of the *Convolvulaceae* and a relative of *C. arvensis,* and we would later see hybridization signals in apple [40]. See [39] for recent literature. Later, on a visit to Madison, Jerry Slightom and I made a phage Lambda library from *C. arvensis* DNA that I took

to Versailles with the intention of purifying and mapping positive clones for sequencing.

This never happened. I became overwhelmed by the complexities of doing research in Versailles. I had been obliged to obtain and maintain my own infrastructure, including a prefab building to use as a lab. The director of INRA, Jacques Poly, had been encouraging, but INRA had not accepted a grant from Agrigenetics to pay the costs of the research. In spite of promises to the contrary, INRA did not provide adequate funding. The grant game in France was fraught with cronyism, entrenched territoriality, and corrupt administrators. I was the American bull in the china shop of French science. Things got even worse with the transfer of French national science funding resources to Europe, increasing paper work and politics. When J. Poly retired, I lost the only moral and financial support that I had at INRA. I was exhausting myself with trivia and attacked on all fronts. I realized that if I wanted to do science, France was not the place for me. The local powers clearly wanted me to leave, and they certainly did not want me working on A. rhizogenes and Ri T-DNA. It seemed unlikely that my ambition to work out how the genes carried by the Ri TL-DNA altered development could be fulfilled in Versailles.

Nevertheless, I decided to ignore the realities and to stay. The reasons were in part personal (an attachment to living in France), but more importantly, at INRA I was free to self-direct my scientific activities. Elsewhere, I would not be able to so hands-on science myself, which I craved. I would have to teach undergraduates and build up a grant-dependent factory of graduate students, postdocs, and technicians. In Versailles, I could have a minimum of internal support in the form of some operating expenses and personnel, allowing me to pursue, albeit on a small scale, subjects of my choosing. I had to lower my ambitions and depend more on collaborations, which turned out to be a source of diversity and inspiration, I tried to keep this collaborative approach organized under the general subject of rhizosphere biology. I decided to leave the Lambda clones in the refrigerator and to let E. Nester's very competent lab in Seattle pursue the hybridization signals in wild type plants. They described the remnants of an ancient Ri T-DNA, which they named the cellular T-DNA (cT-DNA) in *Nicotiana glauca* [35, 38]. I was free to diversify into rhizosphere subjects that were related to the evolution and developmental biology of the Ri T-DNA and parasexual DNA transfer in general, but this required knowing more about the Ri TL-DNA.

3 Ri TL-DNA Structure and Function

A decisive encounter was meeting J. Slightom in Madison, Wisconsin. He proposed to sequence the Ri TL-DNA that was causing the transformed phenotype, using our clones (produced by Lise Jouanin and Françesca Leach) from the Ri plasmid (which contained a second T-DNA, the TR-DNA, which was not causing the transformed phenotype). In the mid-1980s, sequencing on this scale was heroic. In all, J. Slightom sequenced approximately 100,000 base pairs, using the Gilbert and

Maxim technique. To my knowledge, nobody has detected an error in the final 21,126 bp sequence.

My objective was to correlate the DNA sequence with insertional mutagenesis, transcriptional, biochemical, and developmental studies. I obtained everything necessary to do insertional mutagenesis on the Ri TL-DNA from the Fredrick (Fred) Asubel lab in Boston, and I recruited a bacteriologist in Versailles to do the job. The Northern analysis of transcription [28] was done thanks to a collaboration with Mylène Durand-Tardif and Richard Broglie in Nam Chua's lab in New York. See also reference [41]. The missing link was the mutagenesis, which for trivial reasons did not happen in Versailles. Fortunately, it was done in Eugene Nester's lab in Seattle [42]. The key for me was J. Slightom's sequence, which revealed the locations of potential genes (open reading frames or ORFs) and identified protein sequences and transcriptional signals.

An observation made by a visiting student, Hervé Levesque, suggested that there was interesting evolutionary biology hidden in the Ri TL-DNA. He spotted structural similarities among some of the putative proteins encoded by these putative genes (ORFs), allowing their alignment into a gene family. I named them *plast* genes for their effects on developmental plasticity (the propensity to form organs) in the roots and regenerated plants carrying Ri T-DNA. The *plast* genes came in acidic and basic forms, with the acidic on the right and the basic on the left [43]. Interspersed with the *plast* genes were other functions that had seemingly fallen among them. For example, ORF 10 (called *rolA*) encoded a short, basic protein, suggesting a nucleic acid binding protein. ORF 8 was a homologue of the Ti T-DNA gene, IAAM, the first of two genes necessary for auxin synthesis in soil bacteria; intriguingly, it was fused to a *plast* gene. With J. Slightom's further collaboration, the border sequences defining the ends of the Ri TL-DNA were found to be the same as those in the Ti T-DNAs, indicating the same transfer mechanism for the Ti and Ri plasmids [2]. A comparison between the Ri and Ti T-DNAs allowed us to make a case for a common ancestor [43]. These structural studies provided a framework for the ideas I had nurtured since the original observation of the growth of transformed roots.

4 Ecological and Evolutionary Hypotheses

The ecological and evolutionary implications of natural gene transfer by *A. rhizogenes* were difficult to ignore. During the 1980s, I published symposium volume papers and a review outlining some of these hypotheses [1, 25–27], which I attempted to test over the following years in both laboratory and thought experiments. The subject also found its way into the discussion sections of peer-reviewed papers. See for example [4, 43, 44]. The concept of species and their genomes as impervious to outside genetic influence was ingrained in biological thinking (see quote from J. Monod at the beginning of this chapter). DNA transfer from *A. rhizogenes* to a plant and its progeny went against the dictum that acquired characters are not inherited and that the genome is an impenetrable fortress. In contrast, I saw the transformed phenotype as a source of heritable variability that

functioned in diverse species. Under stress, a community could share genetic information of adaptive significance. The T-DNA transfer mechanism and the *plast* genes insured the passage of bacterial T-DNA into fertile plants and their progeny. The structure of the Ri TL-DNA suggested that other unrelated functions were carried along with the diverged *plast* genes, insuring that they would penetrate the genomes and the species of diverse plants. If the direction of transfer were reversed, and plant DNA found its way into the Ri T-DNA, *A. rhizogenes* would provide a genetic bridge between plant species, thanks to the broad spectrum of its interactions. Many seminars to this effect produced positive reactions but a few (particularly in France) elicited anger. Parasexual genetic exchange was taken to be Lamarckian, as opposed to Darwinian. Acquired characteristics, encoded in a T-DNA, were inherited, raising the specter of Lysenko and the dark years of eugenics in the Soviet Union. My seminar attendees were right to feel threatened. The textbook dogma about acquired characters not being heritable was out of date, as was the concept of species. Nature was the original genetic engineer [27].

4.1 DNA Transfer from Plants to Soil Bacteria, Genetic Football

The origin of the genes that *A. rhizogenes* was sending into plants is a fundamental question. I had long thought that they could come from diverse sources, including plants themselves. For instance, the genes in the Ri TL-DNA found in wild type plants could be the original source of the bacterial versions. Necrotic plant biomass enters the soil environment, where it is digested by bacteria. As roots grow, cap cells slough off, liberating their contents into the rhizosphere. DNA persists in the environment [45–47], and bacteria import it; thus, some of this liberated plant DNA could be taken up by bacteria, become incorporated into a T-DNA, and transferred back into plants. Since T-DNA transfer to plants has a wide host range, soil bacteria could provide a genetic link between plants of different species (Fig. 6). I thought of plant and microbial communities as engaged in genetic football [1]. Attempts to demonstrate DNA transfer from plants to bacteria proved to be technically complex, in part due to the presence of DNA encoding marker genes as contaminants in laboratories and as natural constituent of the soil metagenome.

Ultimately, Johann de Vries and Wilfried Wackernagel used a simple and sensitive method for detecting the uptake and incorporation of a DNA from an antibiotic resistance gene in the soil bacterium, *Acinetobacter baylyi* strain BD413 (Fig. 6). The method was marker exchange, in which an intact *nptII* (kanamycin resistance gene) from the source DNA replaces a defective *nptII* in the target DNA through homologous recombination [48]. We used this system (kindly provided by J. de Vries and W. Wackernagel) to documented DNA transfer from six species of plants into *A. baylyi*. (Results were first presented at the European Society for Evolutionary Biology, Barcelona, Spain, 1999.) Sources included crushed leaves, intact leaves, and intact plants in vitro. Transfer was dose dependent and DNase sensitive; the problem of contaminating DNA was resolved by introducing silent mutations in the

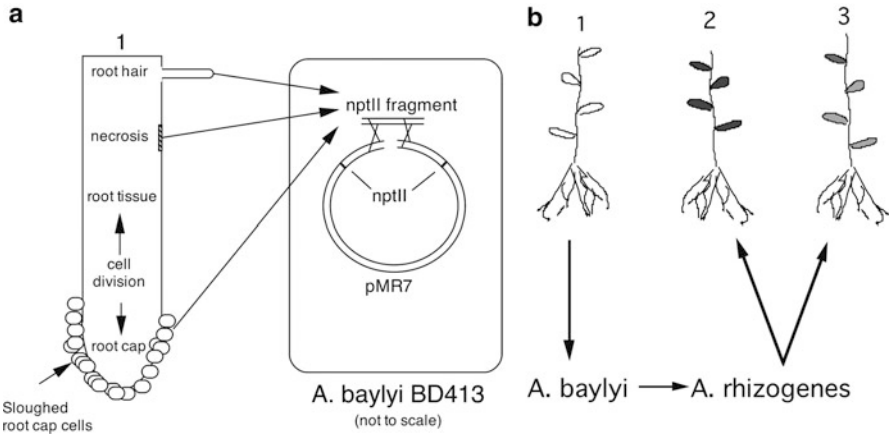

Fig. 6 Parasexual DNA transfer between plants and soil bacteria. (**a**) DNA, released from roots by cap cells, necrotic wounds, and root hairs, is assimilated by *A.* baylyi, where it recombines with homologous sequences through marker exchange [48]. In this laboratory example, fragments of an *nptII* antibiotic resistance gene from the root rescue a deletion in the *nptII* gene carried by plasmid MR7, rendering *A. baylyi* resistant to kanamycin [49]. (**b**) DNA from plant species 1 is transferred (via *A. baylyi* and *A. rhizogenes*) into species 2 and 3 [25, 26, 50]

source plant *nptII,* which were detected in the bacterial target [49]. See references [45, 51, 52].

Once in *A. baylyi*, it was easy to imagine how conjugation could move a DNA sequence into other bacteria, e.g., *Agrobaterium* having a DNA transfer system for plants. However, DNA incorporation in *A. baylyi* depended on homology between the incoming foreign DNA and sequences carried by *A. baylyi*. Homology could be provided by the Ri T-DNA already in the plant host. (The Ri plasmid is maintained in *A. baylyi*, Message and Tepfer, unpublished results.) Thus, DNA transfer in either direction would provide the homology for shuttling other sequences between plant species via *A. rhizogenes*, e.g., those interspersed among the *plast* genes. The direction of the initial transfer could not be known, and it was not important, once the homologous sequences were in place. But what sorts of genes would make this journey back and forth? The opine synthesis genes were transferred to plants from bacteria, but suppose they had come from plants via a pre-established homology? These questions inspired a fresh look at nutritional relationships between plants and bacteria in the rhizosphere.

5 DNA Transfer, Plant-Microorganism Interactions, Opines, Calystegins, and Betaines

The microorganisms that live around roots are nourished by root exudates, root necrosis, and the contents of the cap cells that are sloughed off during root growth [53]. Bacterial catabolism of plant secondary metabolites is adaptive and specific to

plant species, whether the metabolites are encoded in a T-DNA or not. I thought of these generalized opines or nutritional mediators as the currency in the economic system of the rhizosphere. *A. rhizogenes* was closely related to *Rhizobium*, capable of nodulating legume roots and fixing nitrogen in the nodules. J. Tempé identified opine-like substances in the relationship between *Rhizobia* and their legume hosts [14, 54]. Most conveniently, there was a first rate *Rhizobium* lab on the INRA campus in Versailles.

While using high voltage paper electrophoresis and silver nitrate staining to assay for agropine in *C. sepium* transformed by *A. rhizogenes*, I was intrigued by a pair of substances in the roots of both transformed and control plants. They reacted with silver nitrate, like agropine and manopine, and they accumulated preferentially in the underground organs of both control and transformed plants in quantities similar to agropine, so I thought they could be exclusive sources of nutrition for plant-associated soil bacteria. I screened for metabolism of these calystegins, as I named them, in 42 laboratory strains of soil bacteria, and discovered that they were only metabolized by *Rhizobium meliloti* strain 41, but not by the other soil bacteria [55]. This strain was serendipitously in my collection thanks to interactions with the Versailles *Rhizobium* group (J. Denarié, P. Boistard et al.). They had been looking for the genes for nodulation and nitrogen fixation on a large plasmid, pRme41a, so they had marked it with a transposon insertion, and they had cured the host *Rhizobium* of the plasmid, showing that the symbiotic genes were not on pRme41a. I tested these derivatives for calystegin catabolism: the host *Rhizobium* lacking the plasmid did not catabolize calystegins – nor did the transposon insertion [55], which turned out to be in the calystegin catabolism region. The odds of this happening were like hitting a jackpot in the slot machine of laboratory research.

These simple experiments led to the characterization of the calystegin catabolic region on pRme41 [56]. Screening in the subterranean organs of 105 species of plants revealed calystegins in only three species: *C. sepium*, *C. arvensis*, and *Atropa belladonna* [57]. A. Goldman (who had discovered nopaline and octopine) purified the calystegins, and their structures [57] showed that they were novel tropane derivatives that resembled glycosidase inhibitors. Glycosidases are important in cell to cell recognition, among other functions, and they are thus poisons. Thanks to a collaboration with Russell Molyneux and Alan Elbein, the glycosidase prediction proved to be true, and they inhibited seed germination and root elongation. Furthermore, the roots of plants producing calystegins carried bacteria that catabolized them and nonproducing plant roots did not [58]. See [59] for different result.

An ecological interpretation seemed obvious. Plants liberate carbon and nitrogen into the soil in the form of exotic molecules that are catabolized only by soil bacteria that carry special catabolic genes. But calystegins are not just exclusive carbon and nitrogen sources; they are also allelopathic glycosidases that kill animals and plants in the rhizosphere. Thus, the bacterial catabolic genes would not only provide exclusive nutrition to soil bacteria but they would protect the host plant by detoxifying the soil, and (conveniently for spreading them among bacteria) they are carried on a self-transmissible plasmid. The opines and T-DNA transfer were just one facet of a general network of nutritional relationships. Similarities with the

digestive tube in animals were easy to imagine – so were the possibilities for genetic football. But how did nitrogen fixation fit into this picture? Calystegin catabolism was not necessary for nodulation and nitrogen fixation. *R. meliloti* (pRme41) was probably isolated from soil where morning glory had grown recently. (Rhizobia have a saprophytic existence, aside from their nodulation and nitrogen-fixing activities.)

We set out to find another nutritional mediators that might be more directly related to nodulation and nitrogen fixation. We focused on the betaines present in alfalfa seeds in large quantities, thinking that the germinating seeds would liberate betaines and initiate interactions with *Rhizobium* through nutritional selection. We localized betaine catabolism to the *Rhizobium* symbiotic plasmid (pSym), surrounded by genes involved in nitrogen fixation [60]. Mutagenesis showed that in keeping with the functions of neighboring genes, catabolism of the betaine, stachydrine, was required for efficient nodulation [61]. Thanks to the collaboration of Michael Burnet and J. Slightom, the catabolic region was dissected and sequenced, revealing similarities to bacterial genes involved in the detoxification of xenobiotics [62].

We concluded that nutritional relationships are important in nodulation, just as they are in DNA transfer, but that allelopathy can also be involved. Layers of complexity had been added during the coevolution of plants and bacteria. Genes encoding anabolism and catabolism of nutritional mediators were potential hitchhikers on the basic *plast* gene T-DNA vehicle. There was no evidence for DNA transfer in *Rhizobium* symbiosis, but there was no need for it, since that was amply carried out by a sister bacterium, *A. rhizogenes.* The origin of the catabolic and anabolic gene pairs was thus not important, since they could be kicked back and forth in genetic football. More importantly, the work on nutritional mediators provided evidence for the principle [53, 63] that plant/microorganism relations are modulated by nutritional relationships. The anabolic genes (carried by Ri and Ti T-DNAs) are clearly the objects of genetic football in the case of the opines, and other nutritional mediators (e.g., calystegins and betaines) were candidates as well. What other functions could serve as genetic footballs?

6 DNA Transfer, *Phytophthora* Resistance

A colleague in Versailles, J.C. Pernollet, was working on a family of defense-inducing, small proteins, called cryptogeins, in *Phytophthora*, the fungus responsible for the late blight that caused the Irish potato famine in the mid-nineteenth century. They are secreted in large amounts to scavenge sterols. It was thought that plants use these elicitins as indicators of the presence of *Phytophthora cryptogea* and as a signal to turn on their defenses, including the production of antimicrobial metabolites. J.C. Pernollet et al. had determined the amino acid sequence of β-cryptogein from the purified protein [64]. A genetic football experiment was obvious: if the gene for β-cryptogein was expressed in a host plant, it could set off a defense response and protect the host. A postdoctoral fellow, Helène Gousseau, synthesized a DNA sequence that encoded the β-cryptogein protein described by J.C. Pernollet et al. The resulting gene was also mutated to replace a key amino acid,

Fig. 7 Tobacco plants decapitated and inoculated with *Phythophtora* at points shown by *arrows*. *Left*, plant carrying the *crypt* gene encoding β-cryptogein, a fungal elicitor of plant defense responses. Necrosis was stopped, and the plant made lateral branches. *Right*, a wild type control. (Reprinted from Ref. [45]) (Photo D. Tepfer)

Lysine-13 by a Valine, and it was expressed in yeast by another postdoctoral fellow, Michael O'Donohue [65]. When injected into tobacco leaves, the wild type *β*-cryptogein from the synthetic gene produced the same necrotic lesions as the purified natural protein, while (as predicted from earlier work) the mutant induced relatively little necrosis [66]. Expression of the synthetic cryptogein gene and the mutant under a constitutive promoter in tobacco by a visiting student, Catherine Boutteaux, was correlated with varying degrees of *Phytophthora* resistance [44]. The necrosis induced by inoculation of decapitated plants with mycelium or by infection of root systems with zoospores was reduced in the transformants, including the K13V mutant (Fig. 7) [44]. See also [67]. The degree of resistance increased when the foreign gene was in the hemizygous state, which was reminiscent of the increased penetration of the T' phenotype that was associated with hemizygosity in tobacco transformed by native Ri T-DNA (see above).

The mechanism of increased resistance was not constitutive systematic acquired resistance (SAR), because levels of salicyclic acid and PR proteins stayed at basal levels in control and transgenic plants until they were challenged by *Phytophthora* [44]. A working hypothesis was that increased antifungal secondary metabolites were interfering with *Phytophthora* infection. In collaboration with Sumita Ja, we tested this indirectly using other metabolites by expressing the β-cryptogein gene in other plants (See Sect. 9, below.)

We had made a genetic football out of β-crytogein, but could nature have done likewise? Could *Agrobacterium,* coupled with *A. baylyi,* mediate gene transfer between a fungal pathogen and the plant host? Necrosis of *Phytopthora* would liberate fungal genes for elicitins like β-cryptogein into the soil, but they would

have to somehow be incorporated into a T-DNA to be transferred to plants, which would require DNA homology between a soil bacterium, such as *A. baylyi*, and *Phytophthora*. Some bacteria, including *Agrobacterium,* can genetically transform fungi [68, 69], so T-DNAs could be present in the bacterial and fungal genomes that would provide the homologies required for the transfer of genes like β-cryptogein) into *A. baylyi*, where Ri plasmids are be maintained (Message and Tepfer, unpublished results). Conceptually at least, these experiments brought fungi into the genetic football game, leaving moot the question of the origin of the transferred DNAs. Such transfers would take place on an evolutionary time scale, so human intervention was necessary to demonstrate their feasibility in nature, but the elements necessary for the existence of a multidirectional network of genetic links between plants, fungi and bacteria were in identified, and the transfer from a fungus to a plant (with a little help from soil bacteria and laboratory scientists) resulted in increased resistance to the fungal pathogen, showing it could have adaptive significance in nature.

7 DNA Transfer, Plant Development, Polyamines

In the meantime, I had not lost interest in untangling the biochemistry connecting the Ri TL-DNA to the transformed phenotype. One approach was electronic: as data banks filled with DNA sequences and, by extension, amino acid sequences and functions for enzymes and structural proteins, I periodically searched for protein sequence homologues for Ri T-DNA genes, but with no obvious success. A classical chemical approach proved more fruitful. A quick look at the Merck index suggested that more information had accumulated about the biochemicals than about the enzymes that produced them, and chemical structural similarities had led us to the identification of the calystegins as glycosidase inhibitors. Biochemicals are conserved among species, and diverse but related structures and functions have evolved from simple precursors. The transformed phenotype was conserved in numerous dicot species. Logically, the Ri T-DNA genes would function through conserved biochemistry.

I met a polyamine biochemist, Josette Martin-Tanguy (INRA, Dijon, France) at a NATO conference in Copenhagen. Since polyamines are implicated in plant development (e.g., in flowering), and they are ubiquitous in living organisms, we decided to look for possible correlations between changes in polyamine accumulation and the transformed phenotype. A series of papers was the result [29, 36, 70–74], and some of these are discussed below.

In Versailles, we produced T and T' tobacco plants expressing wild type Ri T-DNA or just *rolA* or *rolC* from the Ri TL-DNA. We also provided tobacco plants singly expressing these genes under the constitutive control of the 35S-CAMV (Caluliflower Mosaic Virus) promoter. In Dijon, J. Martin-Tanguy and her student, Daniel Burtin, showed that tobacco plants displaying the transformed phenotype had reduced free polyamines and polyamine hydroxycinnamic acid conjugates, and that this reduction occurred in direct proportion to the severity of the phenotype:

e.g., there was greater reduction of polyamine and polyamine conjugate titers in the T' phenotype than in the T phenotype [74]. Feeding polyamines to plants of intense T' phenotype caused attenuation to the T phenotype [29]. An early step in polyamine synthesis is inhibited by α-DL-Difluoromethylornithine (DFMO), which produced a phenotype in tobacco that resembled the transformed phenotype caused by the Ri TL-DNA, confirming that the inhibition of polyamine accumulation via the ornithine pathway is an essential step in the chain of biochemical events that lead to the transformed phenotype [73].

Another series of experiments concerned plants expressing *rolA,* driven by the wild type promoter. In accord with references [75, 76], we concluded that *rolA* was the major determinant in the T' phenotype. Furthermore, inhibition of the polyamine conjugates, rather than the polyamines themselves, was the biochemical correlate. 35S CAMV *rolA* plants had an extreme T' phenotype, and they were unable to flower [29]. This extreme phenotype could not be attenuated by watering 35S CAMV *rolA* plants with polyamines, because they were deficient in the polyamine conjugates whose accumulation in the top of the shoot occurs prior to flowering in wild type plants. Conjugated polyamines were not available for watering experiments, so P_{35S}-*rolA* shoots were grafted by J. Martin-Tanguy et al. onto wild type plants that had been induced to flower and had accumulated conjugated polyamines in their shoots. The P_{35S}-*rolA* scion flowered, but the flowers aborted, falling off the plant [29] (Fig. 8). This last defect was corrected by watering with putrescine alone. A control wild type rootstock that had not been induced to flower did not restore flowering. A final series of attenuation experiments performed by a graduate student, Li-Yan Sun, using plants expressing *rolA*

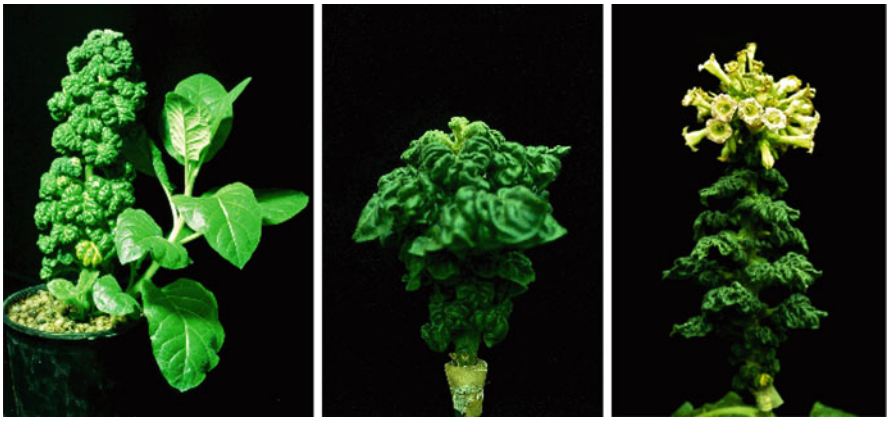

Fig. 8 Attenuation of extreme phenotypes in Xanthi tobacco due to Ri TL-DNA and its *rolA* (ORF 10) gene driven by the 35S CAMV promoter. *Left*, extreme T' phenotype in a plant transformed by wild type Ri T-DNA, with reversion to the attenuated T phenotype in a lateral shoot (see also Fig. 5). *Center*, phenotype due to 35S *rolA* in a shoot grafted onto wild type tobacco that was not induced to flower and had not accumulated polyamine conjugates. *Right*, same genotype as middle plant but grafted onto a wild type plant that had been induced to flower and had accumulated the polyamine conjugates (Reprinted from Refs. [29, 73])

from wild type regulatory sequences, showed a correlation between phenotypic attenuation, reduction in *rolA* transcripts and methylation of sites in the 3' regulatory region [72]. The phenotypic changes associated with transformation by the Ri TL-DNA and *rolA* alone were attenuated by polyamines and polyamine conjugates.

To be transmitted through meiosis, the Ri T-DNA encodes increased plasticity, i.e., root and shoot formation, but increased plasticity is not adaptive if it interferes with fertility. Attenuation is accomplished through homozygosity [29] through segregation of truncated T-DNAs [77], through physiological compensation (by making more polyamines and their conjugates) [29] and by decreasing transcription, e.g., through methylation of the 3' regulatory region [29]. The foreign DNA is thus regulated to insure sexual transmission. Attenuation through homozygosity would select for progeny from self-fertilization and counter select those from out crossing. It should thus drive speciation.

8 DNA Transfer, Biospheres, Flavonoids

Sydney Leach, a physicist friend, suggested that we work together on the origin of life, which seemed like a complex and distant subject until I realized that for the sake of simplicity, life could be defined as DNA and that the origin of life was a problem in DNA transfer. Life was present early in the history of the Earth and all life on Earth that has been examined uses essentially the same genetic code, strongly pointing to a common origin for the life forms we know. These two facts are compatible with, but do not prove panspermia, the ancient hypothesis that life is everywhere. The European Space Agency (ESA) called for proposals to test the plausibility of the dispersal of life through space by attaching life forms known to survive in extreme environments (extremophiles) to the outside of the International Space Station (ISS). While orbiting the Earth, extremophiles would be exposed to conditions encountered in space, the equivalent of an interplanetary voyage in our solar system (albeit with reduced cosmic radiation, due to partial protection from Earth's magnetic field in low orbit). This was the chance to do the ultimate genetic football experiment (Fig. 9), kicking the genetic foot ball into orbit, thanks to ESA and NASA.

I proposed exposing plant seeds in space, because they resist desiccation, radiation, and they have protective seed coats containing flavonoids that stop destructive UV light. We (including Andreja Zalar and S. Leach) prepared for two exposures in space to determine the survival of both unprotected DNA and seeds and to thus test the plausibility of panspermia. Using the UV beam from the synchrotron at the University of Aarhaus, Denmark, we measured short wavelength UV absorption in known and potential UV shields, finding that flavonoids had UV absorption characteristics similar to those of DNA [79, 80]. Preparatory experiments at the DLR (German Aerospace Center) in Cologne, Germany with Arabidopsis (*A. thaliana*) mutants lacking sunscreens showed that flavonoids (and sinapate esters to a lesser extent) were important in protecting seeds from part of the UV spectrum that is

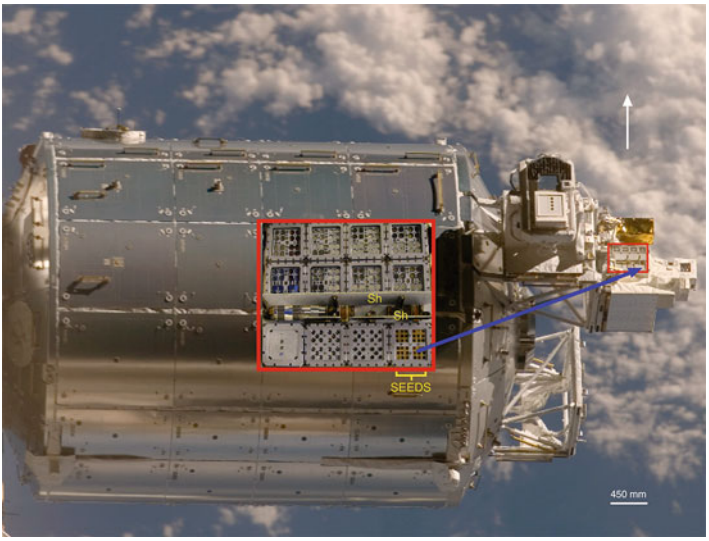

Fig. 9 EXPOSE and SEEDS on the Columbus module of the ISS (Photo courtesy NASA). EXPOSE (*small red box*) is on the EuTEF platform. SEEDS is covered by a protective shutter (closed in this photo, taken prior to the start of exposure). Columbus, the European module, is to the *left* of EXPOSE. Proximal sources of UV and solar wind shadows include Columbus, the other experiments surrounding EXPOSE on the EuTEF platform and the protective shutter, which was perpendicular to the surface of SEEDS during exposure but closed during transport. *Insert*, (*large red box*), position of SEEDS on EXPOSE, with the shutters (Sh) open with seeds on the *lower right*. *White arrow*, direction of flight, except during shuttle docking, when it was inverted 180°. The external dimensions of Expose were 440 mm × 380 mm × 250 mm (Reprinted from Ref. [78])

deleterious to unprotected organisms in space travel [81]. Exposures to simulated space conditions also showed that Arabidopsis and tobacco seeds were many orders of magnitude more resistant than other potential space travelers, including UV-resistant bacterial spores [81].

The first exposure on the outside of the ISS lasted 18 months; 23% of the exposed Arabidopsis and tobacco seeds germinated and produced fertile plants after return to Earth [78]. We concluded that an unprotected Arabidopsis seed could theoretically survive a direct transfer from Mars to Earth, and that resistance was largely due to flavonoids. Furthermore, the survival of a 110 bp fragment of unprotected DNA was detected by PCR. A second experiment on the ISS established an end point for resistance of Arabidopsis seeds to space travel and localized the part of the UV spectrum that was most deleterious (Tepfer and Leach, submitted). Further laboratory experiments on Earth showed that morning glory seeds, which have thick seed coats and are long-lived in nature, were much more resistant to $UV_{245\ nm}$ than Arabidopsis and tobacco seeds, suggesting that larger seeds with more protective coats should survive much longer exposures (Tepfer and Leach, submitted). The lack of morphological mutants in the plants that grew from exposed seeds led us to measure the structure and function of a *nptII* marker gene carried by the plastids in

exposed tobacco seeds. We used both quantitative PCR and marker exchange in *A. baylyi* to show that DNA damage was being circumvented and repaired, and that other targets were more limiting to seed survival, e.g., ribosomes, membranes, and proteins (Tepfer and Leach, submitted).

These experiments were a thinly disguised excuse for doing another genetic football experiment. The use of marker exchange to measure DNA integrity in exposed seeds was a proxy for DNA coming to Earth and entering the biosphere through homologous recombination in a naturally transformable bacterium, such as *A. baylyi*. Marker exchange showed how extraterrestrial DNA sequences (exoDNA) would influence Darwinian evolution on Earth. Fragments of the *nptII* gene survived unprotected exposure to full space conditions; thus, naked or protected DNA from Earth's biosphere could be carried by updrafts into the stratosphere, returning to Earth at a later time and reentering our biosphere through homologous recombination in bacteria such as *A. baylyi*. Mutations acquired in space would thus provide variability for Darwinian evolution. The forces needed to eject seeds from Earth via meteorite impact were too great for seeds to survive, but fragments of seeds did survive in simulations of those ejections [82].

To be efficient, natural transformation of *A. baylyi* requires at least a few hundred base pairs of one sided homology between the incoming and recipient DNA [83], limiting the inward transfer of extraterrestrial DNA to sequences originating on Earth and suggesting that life on Earth is insulated from exoDNA. On the other hand, if DNA transfer into the biosphere were not homology independent, partially degraded DNA and bacteria carried by micrometeorites or defunct extraterrestrial organisms, resembling seeds, could enter Earth's biosphere. Among the mostly unknown microbes, there could be species capable (e.g., when under stress) of incorporating heterologous DNA into their genomes. Alternatively, DNA sequence evolution could be open to exoDNA via universal, homologous sequences like the Ri TL-DNA that would genetically connect distant biospheres. This discussion is relevant to proposals to send humans to Mars (see below).

Nucleic acid transfer through space is a plausible way to explain the past origin of the life we know on Earth. However, the future of our life will be limited by increased heating from the Sun. For life is to survive, it will have to be exported, e.g., in a vector like a plant seed, to exohabitats. Ironically, dispersal away from Earth has already occurred through the contamination of unmanned space probes with UV-resistant bacterial spores [84, 85]. Exospermia (as opposed to endospermia) is thus happening through our space explorations, in a reversal of the directed panspermia hypothesis evoked to explain the origin of life on Earth [86].

If we are directing panspermia, why not do it as best as we can? What are the most resistant life forms that can be sent to exohabitats, and how can their resistance be improved? In the case of seeds, they could be chosen for inherent resistance (e.g., morning glory) and also genetically modified, e.g., to accumulate more of the flavonoid sunscreens [87, 88]. They could also be coated with UV screens and loaded with free-living, beneficial bacteria (e.g., nitrogen-fixing *Rhizobium*). Genetic redundancy in nuclear DNA can be increased in plants by increasing ploidy levels [89]. The redundancy of vulnerable components, necessary for recovery, such as

ribosomes, chloroplasts and mitochondria, might also be improved. The latter two might be genetically modified with high performance bacterial DNA repair systems, like those in from *Deinococcus radiodurans* [90].

Humans are poorly suited for space travel, but they would be excellent vectors for microbial life. A human death on Mars could liberate roughly 10^{14} microbial cells [91]. Seeds and selected microorganisms could be sent to specific exohabitats [78, 85, 92] in a vanguard for human colonization. While seeds might survive space travel and germinate, what would happen to the plants they produce? The deleterious effects of UV on mature plants have been described only on Earth, where atmospheric ozone filters out $UV_{<300\ nm}$. DNA absorbs strongly at wavelengths below 300 nm. Thus, sunlight for photosynthesis must be filtered via external shading, increased inherent filtering, e.g., through increased flavonoids, and through an atmospheric filter, like oxygen and derived ozone. An ideal exohabitat would include enough atmospheric oxygen to protect against UV and to supply mitochondrial respiration.

Voyager I is over 20 billion km away from the sun. Humans are currently engaged in genetic football on a cosmic scale, but time is running out for life on Earth. In roughly one billion years the Earth will be too hot for even microbial life to survive; thus, only 20% of life's tenure on Earth remains for finding another habitat, and at our present pace of self-destruction, humans will be extant and healthy enough to accomplish exospermia for a much shorter period. In the meantime, better UV and radiation resistance in plants could be useful in the event of damage to the ozone layer, increased radiation from nuclear wars or during magnetic pole reversal.

9 Biotechnological Applications

Soon after the initial culture of transformed roots *in vitro*, numerous biotechnical applications became obvious or were proposed by collaborators. It seemed important, while doing the basic research outlined above, to stay connected to the real world, so applications were pursued both in-house and through collaborations. The most obvious of these, the use of the Ri plasmid as a genetic transformation vector for crop plants, was aggressively developed by several competent labs, so my participation was not needed, and my proposal that the transformed phenotype was a better marker than antibiotic resistance was not accepted (Vectors were soon made with Ti T-DNA in which the plant hormone genes were removed.). Applications using the transformed phenotype (e.g., transformed roots and altered root and shoot architectures) were more interesting than genetic engineering, because they led me into unfamiliar subjects. Thus, on a much smaller scale, through seminars and the dissemination of *A. rhizogenes* bacterial strains, we encouraged the use of wild type *A. rhizogenes* and the transformed phenotype as a means of genetically improving plants without resorting to DNA manipulation [27].

Since transformation by *A. rhizogenes* occurs in nature, it is often considered to be natural and not to involve genetic manipulation. Thus, a cottage industry has developed that uses wild type *A. rhizogenes* in clever and inexpensive

biotechnological applications. I find that low budget research is often more inventive and adventurous than the well financed version, because it requires more imagination. Also, it can be done in developing countries, where innovation has a bright future [93]. I also believe that inventions generated in the public sector should belong to the public domain, and INRA is a governmental research institute. Nevertheless, five patents were awarded to us, but not pursued by INRA. All intellectual property described here is now in the public domain. To my knowledge, the only research currently in commercial application is the production of an AM fungal inoculum on transformed roots. See Sect. 9.3.

9.1 Chimeric Plants Through Genetic Grafting

In nature, *A. rhizogenes* presumably produces a transformed, secondary root system. Claudi Lambert, a graduate student, showed that *A. rhizogenes* could root recalcitrant apple root stocks and that the opines were translocated into the wild type plant parts of chimeric apple trees from a genetically transformed root system [94, 95]. See also references [96–98]. We envisioned using genetic grafting to improve edible plant parts, e.g., apples, using genes transferred to the root system, but not to the aerial parts. A transgenic apple tree showed increased root and shoot production, as expected with augmented plasticity.

9.2 Use of Transformed Roots to Determine Cadmium Availability in Contaminated Soils

A Versailles colleague, René Prost, suggested that we use transformed root cultures to measure the availability of cadmium and other toxic heavy metals in soils. Heavy metals are released by mining and manufacturing, from sewage sludges and batteries (to name just a few sources). They are spread through water, wind, human activity and by animals and plants that absorb and concentrate them. Once released, they stay in the environment, from which they are readily taken up by both animals and plants. However, heavy metals can become complexed with soil constituents and thus taken out of biological circulation.

While it is relatively simple to measure heavy metals in the soil, there was no inexpensive and reliable way measure their availability to living organisms (bioavailability), which is a crucial parameter in predicting toxicity. We thus set out with a postdoctoral fellow, Lionel Metzger, to use transformed roots as a living assay for bioavailability. The result was a feasibility study that showed that transformed tobacco and morning glory roots could be used to bioassay Cd availability in contaminated sewage sludges [99]. We also noted that transformed roots in culture take up little space, that they are inexpensive to produce and that they avoid the variable and complex influences of the aerial plant parts, providing simplicity and reproducibility.

The next project was to use what we had learned about Cd uptake and toxicity to stabilize and detoxify extremely polluted waste dumps, where no plants could grow. I coordinated a proposal to the European Union to attempt this reclamation using morning glory, genetically modified to specifically nourish a heavy metal sequestering soil bacterium. The objective was to first stabilize the site against erosion, then to harvest the morning glory as it removed Cd from the soil. (This was before the concept of phytoremediation was invented.) The proposal was not accepted.

9.3 Use of Transformed Roots to Propagate Arbuscular Mycorrhizal Fungi In Vitro

Jacques Mugnier, a colleague at a nearby Rhône-Poulenc lab, used my transformed morning glory roots to grow an obligate, rhizosphere symbiont, an arbuscular mycorrhizal (AM) fungus [100] (Fig. 10). See also [101]. AM fungi are essential for the growth of about 80% of the vascular plants. Hyphae invade root cortical cells, where they exchange minerals (e.g., phosphorus) for energy-rich nutrients from the plant. Hyphae form extensive networks in the soil, complement the host's root system and physiologically connect plants of different species. Attempts had been made to produce their spores in pot-grown plants, but they were stopped for fear of propagating pathogens. Axenic root cultures solved this problem. An inoculum consisting of spores and transformed roots is currently used to produce biological fertilizer for soil remediation in India, thanks to research by Alok Adholeya and his coworkers at TERI University in New Delhi, India [102]. We have used transformed roots to explore similarities between AM fungal and Rhizobial mutualisms [102] in a collaboration with Boovaraghan Balaji and Yves Piché (Université Laval, Québec).

I was intrigued by the possibility that plants of different species could be connected through a common network of AM fungi. I concocted a two compartment model that relied on staining and autoradiography for following ^{23}P transport and uptake into the roots by the fungus. The basal parts of the roots were nourished by a rich, solid medium (simulating the plant) in a small Petri dish inserted in a large Petri

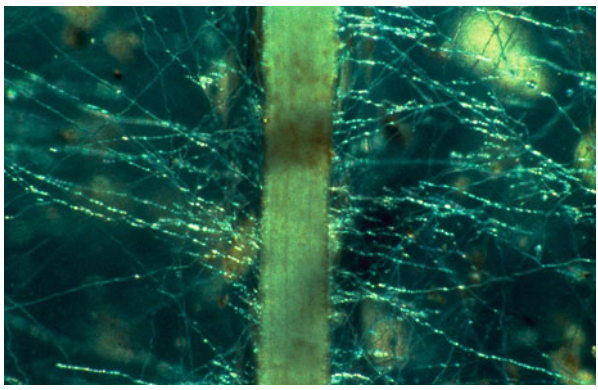

Fig. 10 Root segment, oriented vertically, from a culture transformed by *A. rhizogenes* and propagating hyphae of an arbuscular mycorrhizal fungus, *Glomus mosseae*, running horizontally (Co-cultivation J. Mugnier; Photo D. Tepfer)

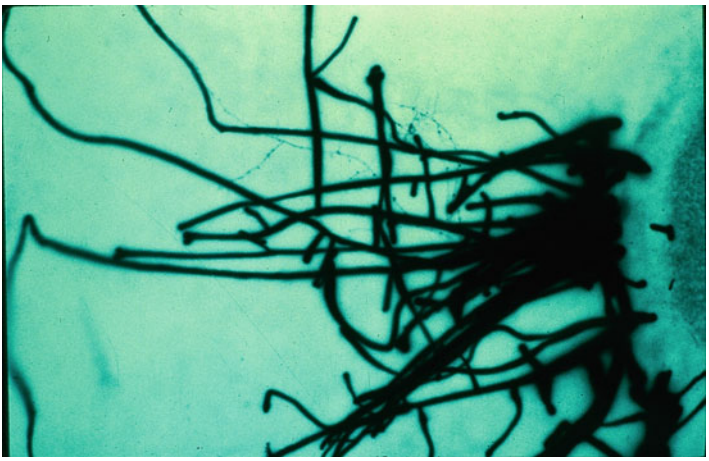

Fig. 11 Autoradiograph of transformed *C. sepium* roots and AM fungal hyphae (*Gigaspora margarita*), labeled with ^{32}P, taken up from the medium in a large Petri dish filled with agar + water. The roots were nourished through their proximal parts by a rich medium in a small Petri dish set inside the large Petri dish. The small dish (formerly on the *right*) was removed to allow for autoradiography, after cutting the roots at its edge. The *thick lines* are roots, Photo and the *thin lines* are hyphae (Co-culture, J. Mugnier. Autoradiography and experimental design, Photo D. Tepfer.)

dish, where the fungal/root interaction took place in a basic agar + water medium. We could monitor the movement of ^{32}P from a point source in the large compartment (simulating the soil) into hyphae and the roots by removing the outer compartment and drying the medium onto filter paper like an electrophoresis gel. Staining of the hyphae and autoradiography revealed the spatial and functional relationships between the roots, the hyphae and ^{32}P (Fig. 11). Transformed roots in vitro could be used to model other rhizosphere relationships, free from the complexity of the soil [16]. The experiment I never did was to connect the roots of two species using two small Petri dishes by AM fungi in a single large Petri dish and to look for chemical connections between them, including DNA transfer (genetic football), perhaps mediated by bacteria harbored by the fungus.

9.4 Use of Genetic Transformation to Alter Secondary Metabolite Production

Early in the adventure of growing transformed roots, I was confronted with a problem of over-supply, which was solved by eating the rapidly accumulating carrot roots (Fig. 2). Compared to normal roots in culture, they had an intense, spicy, carrot flavor with an unexpected hint of pepper. This taste test was the first indication that secondary metabolite production was altered and enhanced in plant tissues transformed by *A. rhizogenes*.

Fig. 12 Red beet roots transformed by wild type *A. rhizogenes* in an early stage of culture with aeration by sterile air pumped through an aquarium stone. Bioreactors are not needed to grow transformed roots. Cultures can be aerated with simple aquarium equipment plus a filter to sterilize incoming air, and the pressure produced is sufficient to remove medium for extraction and renewal. In one such culture, morning glory root density was so high that they had to be sawed to remove them from the culture vessel and the aquarium stone was never found, apparently digested by the roots (Photo D. Tepfer)

Plant cells grown in bioreactors have been used in attempts to produce high value pharmaceuticals. Transformed roots are better candidates for such endeavors because they grow fast, and (unlike cell cultures) they are genetically stable. These advantages became evident when a colleague, Gerard Jung at a nearby Rhône-Poulenc lab grew my *C. sepium* roots in a 30 l yeast fermenter. He produced two kilos (fresh weight) in two weeks, and he showed that they accumulated increased amounts (10x the titers in wild type leaves) of the tropane alkaloid, cuscohigrine [103]. See a simple bioreactor, Fig.12) In general, transformed roots accumulate the metabolites found in normal roots; e.g., red beet roots are a bright red (Fig. 12), but quantitative and qualitative changes take place in transformed roots and plants. An example is given below.

Scented lemon geraniums (*Pelargonium*) are the source of most of the natural rose fragrance in expensive cosmetics. They are also popular houseplants, but they suffer from having few branches and long internodes, a defect corrected by transformation with wild type Ri T-DNA (Fig. 13) They were attractive subjects for exploring the world of fragrance secondary metabolites and shoot system architecture, and when transformed by wild type Ri T-DNA, they produced quantitatively more and qualitatively different monoterpenes, i.e., fragrances [104]. Geraniol increased 2.0–4.4 fold; cineole increased 3.3–13 fold. Other oxygenated monoterpenes decreased. Overall essential oil production increased. The differences were obvious to human volunteers in blind tests (unpublished results). Leaf production increased by a factor of 3–4 times, compared to wild type controls, giving an overall increased production of geraniol of about tenfold [104]. Furthermore, the transformed plants had improved architecture and

Fig. 13 Transgenic scented geranium plants. *Left*, transformed by wild type *A. rhizogenes*. *Right*, wild type control. Internode distance and apical dominance were decreased, improving stature for use as a house plant. Fragrance production was increased (Reprinted from Ref. [104]). See Fig. 16 for the drought stress phenotypes in the same plants (Photo D. Tepfer)

drought resistance (Figs. 13 and 16). I imagined a houseplant perfume mini-factory, tailored by genetic transformation to the user's fragrance preferences. A collaboration with John Sanford extended our results to other scented geraniums, having very different fragrances, but to my knowledge there is no current commercial application.

Secondary metabolites took center stage thanks to visit from S. Ja (University of Calcutta, India). After showing that transformation by *A. rhizogenes* could increase secondary metabolites *Tylophora indica* [105, 106] and *Withania somnifera* [107], we received a grant from CEFIPRA to attempt to go beyond the improvements associated with transformation by Ri T-DNA. Our approach was to use genetic elicitation with the β-cryptogen gene to trick the host plant into perceiving a fungal attack coming from within its cells (see above, Sect. 6) and thus increase secondary metabolite production. We transformed *C. sepium, C. arvensis, T. indica*, *W. somnifera* and Arabidopsis with Ri T-DNA plus β-cryptogein and obtained secondary metabolite increases beyond those due to the Ri T-DNA (Fig. 14). In Arabidopsis, transformation with the *crypt* gene produced increases in the titers of certain flavonoids [87] (This research is described in more detail elsewhere in this volume.).

Transgenic mimicry of pathogen attack was thus correlated with increased growth and secondary metabolite accumulation in five species for four classes of medicinal substances. These results again suggested that the resistance to *Phytophthora* associated with the β-cryptogein gene in tobacco [44, 67] was due at least in part to increased antifungal metabolites, and they provided a further example of the possible adaptive significance of genetic football. But how does it work? One simple hypothesis is suggested by the role of polyamines in producing the transformed phenotype. Polyamine accumulation is reduced by Ri TL-DNA, which could shift carbon allocation into other anabolic pathways, e.g., for antifungal and medicinal substances.

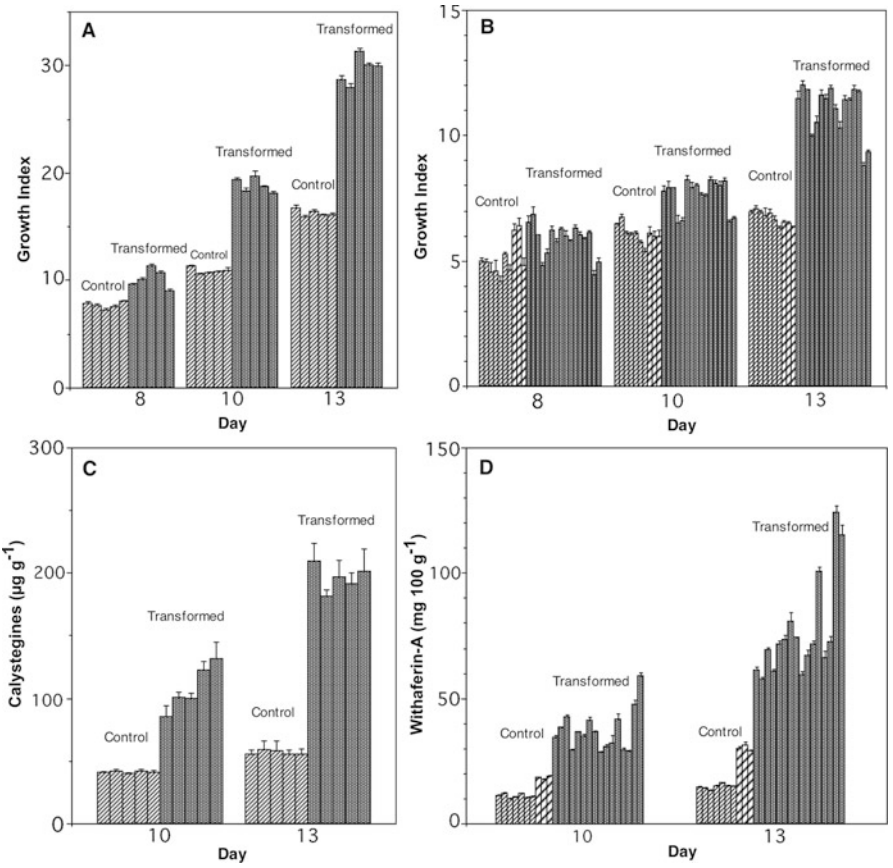

Fig. 14 Effects of genetic transformation with the *crypt* gene on growth and secondary metabolite production. The *crypt* gene was introduced into *C. sepium* (**a**, **c**) and *W. somnifera* (**b**, **d**) using *A. rhizogenes* LBA9402. Growth (**a**, **b**) and secondary metabolite accumulation (**c**, **d**) were recorded over time. Bars represent individual root clones from independent transformation events: dark hatching, roots containing *crypt* and Ri T-DNA; bold hatching, roots containing just Ri T-DNA produced by wild type *A rhizogenes*, carrying the *crypt* gene construct, but which did not receive the *crypt* gene from the pBin19 binary vector; light hatching, roots produced with wild type *A. rhizogenes* (Reprinted from Ref. [87])

9.5 Changing Root and Shoot Architecture

The effects of Ri TL-DNA genes on growth and development have been the subject of numerous studies. A comprehensive review is outside the scope of this chapter, but to give a few examples: in our hands the promotion of annualism and reduced apical dominance was due to *rolC* in carrot (Fig. 15) and Belgian endive (*Cichorium intybus*) [77]; extreme dwarfing was associated with *rolD* in carrot [77]; extreme wrinkling and dwarfing were attributed to *rolA* in tobacco

Fig. 15 Changing phenotype in carrot with wild type Ri T-DNA and its *rolC* gene. (**a**) *Left*, plants transformed by wild type Ri T-DNA, showing reduced apical dominance, flowered as annuals and produced diffuse root systems, instead of carrots; *right*, wild type controls, regenerated from wild type root cultures were biennials, and they produced normal carrots. The plants are of approximately the same age. The difference in size is probably because the products of photosynthesis were stored in carrots in the controls, but reinvested in making leaves in the transformed plants. (**b**) Root system of a plant transformed by *rolC* (ORF 12) from the Ri TL-DNA, showing reduced apical dominance. (**c**) Shoot system of the same plant, showing reduced apical dominance and annual flowering (Photos D. Tepfer)

(Fig. 8) [72], and wild type Ri T-DNA caused reduced apical dominance and internode shortening in numerous species (Figs. 3, 5, 8, 13, and 14).

A grant from the Rockefeller Foundation revived a long-standing, personal concern about climate change and drought resistance. Global warming was upon us, and improving root system architecture was one approach to adapting crop plants to changed access to water. Nonirrigated, upland, rice was the chosen target because of the vulnerability of subsistence farmers in developing countries to even small climate changes. Experiments using scented geraniums grown in the greenhouse in columns of sand, with water and nutrients injected either from the top or from part way down the side, showed that plants transformed by wild type Ri T-DNA responded better to water limitations than the wild type by making use of the expected increased plasticity of their root development when the source of moisture was lowed in the column of sand (Fig. 16). The experiments were done in a large, asymmetrical greenhouse that had evenly distributed

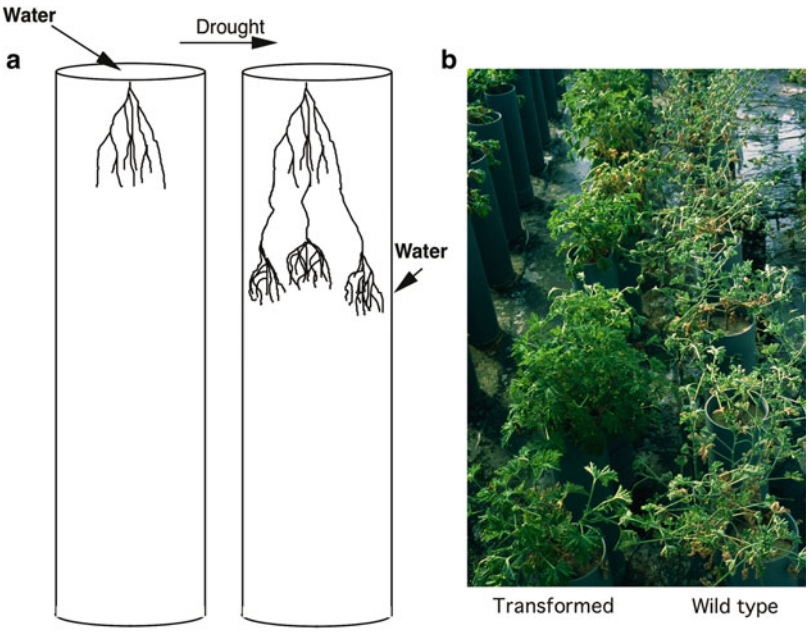

Fig. 16 Resistance to simulated drought in scented geranium transformed by wild type Ri T-DNA. (**a**) System for simulating drought in a column of sand in PVC drainage pipe (unpublished results). *Left*, prior to drought, water plus mineral nutrients were periodically provided through a capillary at the base of the plant. (Aerial parts are not shown.) To simulate drought, the capillary was inserted *lower down* in the column. Root growth and architecture were revealed by lifting the PVC pipe, which liberated the root system intact. Plants responded to drought by sending down deep roots that developed profuse branches at the lowered source of moisture. This adaptation was faster and more efficient in the transformed plants. (**b**) Experiment in the greenhouse part way through drought simulation, with the transformed plants on the *left* and the wild type on the *right*. The transformed plants adapted their root systems to the drought conditions and stayed green. The wild type plants did not adapt and had turned brown by the end of the experiment (Photo D. Tepfer)

light exposure. Unfortunately, a windstorm damaged the greenhouse, which was not repaired because INRA had other plans for it. We were given a much smaller space with uneven lighting, and the experiment could thus not be repeated and published.

However, the real goal was to produce phenotypic changes in rice root systems, and for this we were fortunate to collaborate with Michael Davey et al., who showed that our 35S CAMV *rolA* construct was capable of changing phenotype in rice. The phenotype was similar to that described in tobacco transformed with the same gene [29], with extreme leaf wrinkling and severe alterations of growth and architecture in both shoots and roots. Unfortunately, the grant was not renewed, but we showed that at least one of the Ri TL-DNA genes produced a phenotype in rice that recalled to the one we had described for dicot species.

As I write this, 15 years later, I am discouraged by the lack of progress in reducing the human activities that contribute to climate change, and I continue to lament the potentially beneficial scientific and biotechnological opportunities that were missed due to petty, self-interested political controversies over genetically modified plants. It was thus pertinent to evaluate the importance of genetic football in biosafety.

9.6 Biosafety

The genetic football model predicts the transfer of plant DNA to bacteria. We observed this in the laboratory, but unpublished attempts to evaluate its occurrence in hydroponics and in the soil were thwarted by the presence of antibiotic resistance markers, e.g., *nptII,* in wild bacteria. We therefore made three silent mutations (creating a new restriction site) in the part of the incoming *nptII* that replaces the deletion in the resident *nptII* in *A. baylyi*, restoring *nptII* activity. It was thus possible to unequivocally demonstrate DNA transfer from plants to bacteria by cutting and sequencing a PCR product from the rescued *nptII*, thus proving that bacterial sequences had come from the plant and not from a bacterial or DNA contaminant. We used this system when we showed DNA transfer from the roots and leaves of transgenic plants in the laboratory [49]. See Sect. 4.1.

We choose a novel environment for doing the experiment under more natural conditions: the gut of the tobacco hornworm, *Manduca sexta*, a very large and beautiful insect larva that consumes tobacco plants with gusto and produces copious feces. The marker exchange assay seemed suited for detecting DNA transfer, since it does not rely on intact DNA sequences, and we used (among others) the multi-copy DNA source employed in the seed experiments in space: a tobacco chloroplast insertion of *nptII*, present at about 5,000 copies per leaf cell, allowing flooding the horn worm gut with *nptII*. The hornworms consumed transgenic tobacco plants that had been sprayed with *A. baylyi* containing the deleted *nptII.* We were unable to detect DNA transfer, probably due to high DNAse activity in the hornworm intestine [108]. The follow-up experiments were to take place in the soil and hydroponic microcosms, but personnel and funding difficulties prevented further research.

We nevertheless showed that tobacco hornworms are not obvious intermediates in the game of genetic football.

9.7 HIV Vaccine

The most ambitious biotechnical application of DNA transfer was an attempt to express a viral antigen in plants in the hope of eventually producing an oral AIDS vaccine in collaboration with Anna Kostrazk (a shared graduate student), Simon Wain-Hobson and Monica Sala (Pasteur Institute), and with Tomasz Pniewski (Polish Academy of Sciences). Development of an AIDS vaccine and vaccines based on the expression of antigens in plants are both distant goals. We reasoned that while immediate success was unlikely, in the name of future advances it was important to make a preliminary attempt. Oral tolerance is an inherent problem in vaccines produced in plants, largely because plant secondary metabolites can both elicit oral tolerance and serve as adjuvants. We used the Hepatitis B virus as a model and expressed HBsAg in tobacco. Dried leaves were fed to mice to assay for immune responses and oral tolerance. We concluded that secondary metabolites in plants can act as adjuvants, boosting the immune response, but that their levels needed to be kept low to avoid oral tolerance [109].

The significance of these experiments for genetic football recalled our experiments with the β-cryptogein gene: elicitation of a host defense response was obtained by expressing a gene, encoded in the pathogen, that serves as a signal to turn on the host's defenses. In this case the pathogen was a virus, rather than a fungus, and the genetic host was a plant, which was eaten by a mammalian viral host. These experiments illustrate another level of possible complexity in the genetic football game, and they make me wonder if genes encoding antigens might be subjects for genetic football.

10 Conclusions

It seems like a long way from natural genetic transformation by *A. rhizogenes* to sending seeds into space to find a new home for life, but looking back, the long and circuitous path can be explained by the cast of chemists, physicists, and biologists who visited my parents' house when I was growing up. Starting with a list of interesting characters, the scenario wrote itself, aided by serendipity, setbacks, and desperate attempts to find funding that forced me to take new directions and collaborations. The admonition that I keep my hands in my pockets when visiting my father's lab set off a desire to do hands-on research, which is still unquenched. The frustrations of managing a lab in a hostile environment and the distractions of writing grant proposals, reports, reviewing papers, etc. only contributed to the frustration. Fortunately, the last two years of the space project were spent working blissfully alone, using my accumulated equipment and supplies. I happily did all of

the experimental work after the seeds came back from space [78] (Tepfer and Leach, submitted), confirming my belief that the hands nourish the mind.

My great luck was having eclectic collaborations and a small, faithful core of collaborators in Versailles. The result is a view of genetics, ecology, and evolution that pokes holes in the concept of species, and fits into the general discussion of horizontal gene transfer (HGT), which is coming into general acceptance with discoveries of its occurrence in the animal kingdom [110]. Genetic football is based on a few facts gleaned from experiments constrained by lack of tools and knowledge. Some experiments were done in thought only, and the DNA transfers described here were facilitated by human intervention, but they show that genetic footballs can be passed across species and kingdom boundaries and even kicked long distances through space. Parasexual DNA transfer does occur in nature, and the basic protagonists might not be genes but rather protein functional domains, encoded in nucleic acids, which is why the term horizontal gene transfer should be changed to horizontal DNA transfer. It is still too soon to use amino acid sequences and protein domain functions to reliably reconstruct past parasexual DNA transfers, but as computer modeling and protein biochemistry improve, such an approach could become viable. In the meantime, there are many laboratory experiments to do in real time, e.g., using transformed roots and bacteria growing in bioreactors and in multi-compartmental rhizosphere models to follow markers encoded in DNA and exchanged on a human time scale.

10.1 A General Model for the Role of DNA Transfer in Ecology and Evolution

Bacteria send DNA into plants, but they also receive DNA, e.g., from plants, from their environment. Plant to plant DNA transfer is accomplished through bacterial intermediates, but for the transferred DNA to have evolutionary significance, it must enter a germ line, which in plants means forming a fertile shoot from the cell that received the foreign DNA. This developmental regeneration is accomplished through *plast* genes, carried by the Ri TL-DNA, and these genes have diverged to be functional in many different species. Part of their action is through changes in polyamine accumulation. Plant DNA enters the microbial metagenome through soil bacteria, e.g., *A. baylyi*, which are adept at incorporating foreign DNA, and then it spreads through the bacterial community, e.g., via conjugation, eventually finding its way into a T-DNA, at which point it can be transferred back into the plant community by *A. rhizogenes*. Fungi [111, 112] and even animals [110] participate in this horizontal DNA transfer. There are interesting rules and constraints in this genetic football game [1, 26], and nutrition is an important driving force. Plants are the primary producers of biological energy. They synthesize complex secondary metabolites that can specifically nourish rhizosphere bacteria carrying genes to catabolize them. These nutritional mediators are the currency in the economics of parasexual

DNA transfer, and it takes a catabolic key to unlock their energy. The production of some of them is encoded by genes for opine synthesis, found in soil bacteria and transferred to plants via *Agrobacterium*; others are currently encoded in the plant genome (e.g., calystegins and betaines), but who knows – maybe they were or will become the objects of DNA transfer?

Besides nutritional mediators, other candidates for transfer include DNA sequences that alter development (branching, flowering, root geotropism, etc.) and genes that confer resistance to pathogens. The gene is not necessarily the fundamental functional unit, since through recombination (e.g., in *A. baylyi*) functional domains in homologous protein-encoding DNA sequences can be switched. In a multidirectional genetic exchange system, there is no way to know the origin of a DNA segment, since the genetic code is essentially the same in all of our life forms and DNA spreads horizontally, perhaps explaining the use of a similar genetic code in all organisms tested so far. The biosphere thus resembles a mosaic, with species lines blurred through the sharing of genetic information from all life forms.

And beyond our biosphere? Of the extremophiles tested so far, seeds are impressively adapted to conserving DNA integrity. Something like a seed might have brought life to Earth – perhaps including multiple life forms, explaining the appearance of the Archea, Bacteria, and Eukaryota at the root of the tree of life [92]. Looking into the future, seeds could serve as vectors for disseminating multiple life forms away from Earth. Only about 20% of life's tenure on Earth remains, and humans will be present and capable of dispersing life through space for only a small part of that. In the meantime, biological communities will adapt to environmental stresses by sharing DNA across species and kingdom boundaries.

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