The Ti-plasmid and Plant Molecular Biology

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ABSTRACT

In 1907, Smith and Townsend identified *Agrobacterium* as the causative agent of crown gall, the most common form of neoplasia in plants. Armin Braun, elaborating on Smith's idea about infectious plant cancer, predicted during the early fifties that *Agrobacterium* transfers a Tumor Inducing Principle (TIP) into plants that incites the proliferation of crown gall tumors by triggering the autonomous synthesis of plant growth hormones auxin and cytokinin. Also in 1970, Morel's group in France suggested that TIP is an *Agrobacterium*-derived DNA that specifies the production of unique compounds in crown galls termed opines that are characteristic for and catabolized by the tumor-inciting bacteria. That the TIP is indeed carried by a large tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* was demonstrated in 1973 by my research group in Gent, Belgium. We and others later showed that TIP corresponds to a segment of the Ti plasmid which is transferred by agrobacteria and stably integrated into the nuclear genome of plant cells. The transferred DNA (T-DNA) was found to encode eukaryotic genes required for the production of opines and plant hormones in crown galls. Inactivation of the T-DNA encoded oncogenes involved in hormone synthesis allowed us in 1981 to obtain fertile transgenic plants showing stable Mendelian inheritance of a T-DNA encoded opine synthase gene. These results opened the way to broad range exploitation of T-DNA in plant transformation, physiology and genetics.

Agrobacterium and the Tumor-Inducing Principle

After obtaining an undergraduate degree in zoology (1957), I (J. Schell) studied microbiology and taxonomy in Prof. De Ley's laboratory in Gent before proceeding on to Prof. K. C. Winkler's laboratory at the State University of Utrecht to finish both my PhD thesis and my formal training in microbiology. This was the blossoming period of bacterial phage genetics. Thus, I decided to learn more about phages λ, P1, T2, T3, host restriction-modification systems and circular DNA species in bacteria as a

postdoctoral fellow in the laboratories of Prof. W. Hayes (Hammersmith Hospital, London), A. Weissbach and J. Hurwitz (NIH and Albert Einstein College of Medicine), and L. Siminovitch (University of Toronto). The emergence of molecular biology and the advent of recombinant DNA techniques found me again in Belgium, where I became a professor and director of the Laboratory of General Genetics at the University of Gent in 1967. Based on my basic zoology education and experience in bacterial and phage genetics, I started to work with the plant pathogenic *Agrobacterium* that was already well-known as the causative agent of plant tumors. Together with my colleague and friend, Marc Van Montagu, and a small group of enthusiastic students, including initially M. Holsters, M. Zabeau, N. Van Larebeke, I. Zaenen, and G. Engler, we jointly surveyed and verified the data available in 1969 on *Agrobacterium*, plant tumors and transformation. At that time, crown gall was still considered as a potential model for cancer research as the etiology of animal and plant tumors appeared to be similar. A supporting argument for this was the experiment of the Danish oncologist, Carl O. Jensen in 1910 who successfully transplanted bacterium-free red mangel crown gall tumor tissues onto white sugarbeet stems. In particular, the American plant pathologist, Erwin F. Smith devoted his life work to verify a relationship between crown gall and cancer. His ideas were followed by Armin Braun, who between 1941 and 1978 published many seminal observations concerning the possible mechanism of *Agrobacterium*-induced tumor formation in plants.

Braun wished to resolve the controversy raised by Riker and Berge in 1935 who stated that primary and secondary crown gall tumor formation results from a continuous stimulation of plant cells by agrobacteria, rather than from stable neoplastic transformation of normal cells to tumor cells. In his experiments, Braun used an observation of Riker (1926) who recognized that crown gall tumor formation occurs only in a narrow window of temperature between 26 °C and 28 °C. Braun demonstrated that the incitement of crown galls by *Agrobacterium* requires a wounding of plants and subsequent wound healing for at least 24 to 48 h. Furthermore, he found in 1947 that the exposure of plants to 32 °C at any time within four to five days after *Agrobacterium* infection inhibits tumor formation. Based on these observations, Braun (1950) proposed that *Agrobacterium* can cause a permanent change in the growth of plant cells by temperature-sensitive transmission of a Tumor Inducing Principle (TIP) which is responsible for the neoplastic transformation. Braun (1959) also repeated Jensen's experiments with clonal, bacterium-free crown gall tissues demonstrating that plant tumors are indeed transplantable, as well as that neoplastic transformation is due to a stable genetic alteration of transformed plant

cells. He noticed that different bacterial strains incite tumors with a characteristic morphology, e.g., that strain B2 incites compact tumors in contrast to strain T37 which leads to the formation of shooting teratomas (Braun, 1948). On the eve of the discovery of cell division promoting cytokinins, Braun and Naf (1954) and Braun (1956) reported that crown gall tumors overproduce auxin (the old leptohormone of Haberlandt) as well as cytokinesins (i.e., cytokinins) which were proposed to be N^6-substituted hypoxanthine derivatives. Braun thus demonstrated that crown gall tissues synthesize auxin and cytokinin, and that they can be maintained indefinitely in hormone-, and bacterium-free axenic cultures *in vitro*. The TIP was therefore defined more precisely by Braun as a hypothetical principle which is required for the maintenance of cell division activity of crown galls via the production of plant growth factors.

Further suggestions about the nature of TIP were derived from the work of French chemists, Lioret (1956) and Morel (Menagé and Morel, 1964; Goldman *et al.*, 1969), who found that crown galls produce unique arginine derivatives termed opines. Petit *et al.* (1970) demonstrated that the chemical nature of these opines, such as octopine and nopaline, strictly depended on which type of *Agrobacterium* strain was used for tumor induction. They also showed that the opine synthesized by a crown gall is specifically catabolized by the bacterial strain which incited the tumor. Together, these data suggested that Braun's TIP might be bacterial DNA which, in addition to carrying genetic information required for oncogenic plant hormone production, would also be responsible for directing the synthesis of specific opines in crown gall tumors.

An additional line of evidence indicating that TIP could be a transmissible bacterial DNA, was provided by the Australian microbiologist, A. Kerr. His experiments were based on the early observations of Hendrickson *et al.* (1934) and Locke *et al.* (1939) who isolated an avirulent derivative (A66) of *Agrobacterium* strain A6 and found that co-inoculation of the avirulent and virulent strains in different positions along the stem allowed the avirulent A66 strain to incite tumors. In 1953, Klein and Klein also reported on the transfer of tumor-inducing ability to avirulent agrobacteria. However, Kerr (1969, 1971) was the first to use properly marked bacterial strains for co-infection of plant wound sites and thereby unequivocally demonstrated the transfer of the virulence trait between pathogenic and non-pathogenic isolates of *Agrobacterium*. Later, together with ours (Genetello *et al.*, 1977) and J. Tempé's group in France, Kerr *et al.* (1977) also demonstrated that the transfer of virulence traits between agrobacteria is induced by the opines present in crown galls incited by virulent agrobacteria.

The Discovery of Tumor-Inducing (Ti) Plasmids

In the early 1960s, little attention was paid to the crown gall problem in plant research. Between 1965 and 1974, L. Ledoux reported on the direct transformation of many animal and plant organs with exogenous DNA. Thus, the resolution of the crown gall problem seemed to be very simple to many researchers who accepted the view that nucleic acids could be freely taken up and expressed by plant cells. In fact, Braun's TIP was soon identified as bacterial chromosomal and phage DNA, as well as infective RNA of various size and origin. Parsons and Bearsley reported in 1968 that temperate *Agrobacterium* phages, such as PS8 present in axenic crown gall cultures, may play a role in tumor induction. Because of our experience in phage genetics, we examined this hypothesis. We identified numerous inducible lysogenic phages closely related to phage Ω of Bearsley (1955), as well as defective non-plaque-forming prophages, which could only be observed under the electronmicroscope, in various *Agrobacterium* strains. The phage isolates were classified using virulent and avirulent *Agrobacterium* hosts as well as by detecting DNA sequence homology by filter hybridization. Although certain results obtained in collaboration with others suggested that some phage DNAs hybridized with crown gall DNA (Schilperoort *et al.*, 1974), we were unable to find a clear correlation between the presence of common phages in virulent *Agrobacterium* strains and their absence in non-pathogenic avirulent isolates (Schell, 1975). Despite these negative results, our studies of prophage DNAs led to the recognition that *Agrobacterium* contained one or more large circular plasmids that could be separated from the chromosomal and phage DNAs on alkaline sucrose gradients and purified by ethidium bromide-cesium chloride dye-buoyant density centrifugation. In addition to these separation methods, we characterized the plasmids and defective prophages by electron microscopy (EM), assayed for Ω-type prophage content and pathogenicity of many different *Agrobacterium* species, including virulent strains of *A. tumefaciens* (B_6, B_2A, 11158, TT-111, A_6, 396, 3/1 and 925), *A. rubi* (TR-2), *A. rhizogenes* (Kerr 38) and *A. species* (0362), as well as avirulent strains of *A. radiobacter* (S1005, TR1, 4718, 8149, 417, $M_{2/1}$) and *A. species* (0363). Ω-phage was found only in *A. tumefaciens* B_6 and B_2A, but not in other virulent strains, whereas defective prophages were detected by EM e.g., in the avirulent *A. radiobacter* strains TR1 and 8149, but not in some virulent strains. Thus, these data showed no correlation between pathogenicity and the presence of phages in *Agrobacterium*. On the contrary, our analysis revealed that all virulent strains contained a large plasmid which was absent from avirulent agrobacteria.

To demonstrate that a large plasmid carried the TIP defined by Braun, we obtained isogenic avirulent derivatives of virulent *Agrobacterium* strains. First, we examined the *A. tumefaciens* strains IIB and IIBNV6 provided by Braun and found that the crown gall-inducing IIB strain carried a large plasmid in contrast to the isogenic avirulent IIBNV6 isolate. At about this time, Hamilton and Fall (1971) reported that *A. tumefaciens* strains C58 and Ach-5, in which we also detected large plasmids, lost their virulence when grown at 37 °C. We grew these strains for five days at 37 °C and, after plating, tested 150 independent colonies for tumor-inducing ability: all of them were avirulent! Twelve of these avirulent isolates were tested for plasmids with the above described methods and we found that all had lost their large plasmids. Similar observations made with strain C58 supported our conclusion that the crown gall-inducing ability was indeed carried by a large plasmid which was therefore named tumor-inducing (Ti) plasmid. Following these experiments, we also assigned other genetic markers to the Ti plasmids. Kerr and Htay (1974) found that avirulent *A. radiobacter* strains 84 and S1005, which carried no plasmid, produced a bacteriocin termed agrocin 84 which efficiently killed the crown gall-inducing *A. tumefaciens* strains, but not their avirulent derivatives. As we knew that the loss of virulence was due to the loss of Ti plasmids, we isolated and tested an agrocin 84-resistant derivative (B6S3) of virulent *A. tumefaciens* strain B6. The B6S3 strain proved to be non-oncogenic and contained no Ti plasmid. During our phage studies, we had isolated a particular phage, AP1, which formed plaques only on a Ti plasmid cured avirulent strain C58C9, but not on its virulent Ti plasmid containing derivative C58. This observation showed that genes responsible for the AP1 phage exclusion were also encoded by the Ti plasmid. In collaboration with R. A. Schilperoort (University of Leiden), we tested the model proposed by Petit (1970) in the group of G. Morel and J. Tempé, which predicted that the TIP (i.e., the Ti plasmid) was responsible for the production of specific opines in crown galls, as well as for the degradation of opines by the tumor-inducing bacteria. This group in France showed that strain B6 induces octopine synthesizing compact tumors, whereas C58 incites shooting teratomas producing nopaline. Indeed, it was found that B6, but not its Ti plasmid cured derivative B6S3, catabolized octopine but not nopaline, whereas strain C58 grew on nopaline but not on octopine, whereas its avirulent derivative C58C9 could grow on neither. The Ti plasmids in these strains thus had to encode specific genes for both opine biosynthesis in crown galls and opine degradation in bacteria, and had a different specificity in B6 and C58. Therefore, the agrobacterium strains could be simply classified based on the type of opine synthesis and degradation genes carried by their Ti plasmids.

To unequivocally demonstrate that all these traits were indeed encoded by the Ti plasmid, we exploited Kerr's technique demonstrating the transfer of crown gall-inducing ability between properly marked, but otherwise isogenic, virulent and avirulent derivatives of *A. tumefaciens* strains B6 and C58. Upon mixed infection of tumors, the transfer of virulence from strains B6 and C58 correlated with the transfer of Ti plasmids to the cured B6S3 and C58C9 strains, which also gained the properties of pathogenicity, AP1 phage exclusion, agrocin 84 sensitivity and opine catabolism characteristic for B6 and C58.

In 1973, I reported these studies at a NATO Symposium on "Genetic Manipulation with Plant Materials" in Liège and the data were subsequently published by Schell (1975), Van Larebeke *et al.* (1974, 1975), Zaenen *et al.* (1974), and Engler *et al.* (1975), as well as together with R. A. Schilperoort by Bomhoff *et al.* (1976), and independently confirmed by Watson *et al.* (1975). The significance of these reports might have remained unnoticed by the plant research community which was busily testing Ledoux's DNA transformation data. However, in 1974 at a congress in Szeged (Hungary), G. P. Rédei (1976) provided the ultimate genetic evidence that transformation with bacterial DNA in Ledoux's experiments did not lead to complementation of the *Arabidopsis* thiamine mutants, as Ledoux had reported (1974) and F. P. Lurquin (1976) demonstrated that Ledoux's transformations did not resultd in the integration of exogeneous DNA into either cytoplasmic or nuclear genomes of plants. Thus, the problem of plant transformation was placed in the focus of general scientific attention and the Ti plasmid evolved to be a chief candidate as a potential transformation vehicle, in addition to some plant DNA and RNA viruses (for review see Howell, 1982).

Identification of the Transferred DNA (T-DNA)

The years of discoveries, 1971–1975, provided us with basic molecular biology tools, including DNA reassociation kinetic analysis, restriction endonucleases, DNA fragment separation by gel electrophoresis, labeling of nucleic acids with ^{32}P, detection of nucleotide sequence homologies by Southern blotting and hybridization, and cloning in plasmids and DNA sequencing (Nathans and Smith, 1975; Southern, 1975; Sinsheimer, 1977). Since all earlier DNA hybridization data were proven to be artificial (Schilperoort *et al.*, 1974; Chilton *et al.*, 1974a; Eden *et al.*, 1974) and the Ti plasmids were found to carry the TIP, recurrent efforts were devoted to detect the presence of *Agrobacterium*-derived DNA in crown galls. However, using the whole Ti plasmid as a probe in reassociation kinetic analysis,

Chilton *et al.* (1974b) and Merlo and Kemp (1976) were unable to detect the integration of Ti plasmid in crown gall DNA. A more detailed analysis using individually isolated, and later cloned, restriction endonuclease fragments of the Ti plasmid however demonstrated that DNA from a well-defined and conserved region of octopine- and nopaline-type Ti plasmids was present in different crown gall tumors (Chilton *et al.*, 1977, 1978a; Depicker *et al.*, 1978). To determine the boundaries of transferred DNA (termed thereafter T-DNA), the Ti plasmids were mapped by restriction endonucleases using different cloned fragments as probes in Southern hybridizations with crown gall DNAs (Chilton *et al.*, 1978b; Depicker *et al.*, 1980; Lemmers *et al.* 1980; Thomashow *et al.*, 1980; De Vos *et al.*, 1981). Cellular localization and reisolation of T-DNA fragments from crown gall tumors subsequently demonstrated that the T-DNA was covalently linked to plant nuclear DNA and flanked within the Ti plasmids by two imperfect 25-bp repeats (Chilton *et al.*, 1980; Willmitzer *et al.*, 1980; Yadav *et al.*, 1980; Zambryski *et al.*, 1980). Hybridizations of crown gall RNAs with T-DNA fragments also revealed that the T-DNA carries genes that are transcribed to poly A^+ mRNA by RNA polymerase II in plant tumors (Drummond *et al.*, 1977; Gurley *et al.*, 1979; Willmitzer *et al.*, 1981).

To precisely map and determine the function of T-DNA encoded genes, the transcript mapping and DNA sequencing methods were coupled with suitable insertional mutagenesis techniques allowing the modification of these genes in *Agrobacterium*. To perform mutagenesis using site-specific recombination, it was necessary to identify the mutant Ti plasmids after conjugation into a cured recipient strain. As Ti plasmid transfer could be previously achieved only in crown gall tissues obtained by mixed infection with donor and recipient strains, bacterial genetic techniques were developed to establish an efficient conjugation transfer of Ti plasmids *in vitro*. The observation of Kerr *et al.* (1977) and Genetello *et al.* (1977), which shows that Ti plasmid conjugation could be specifically stimulated by opines (e.g., by octopine for B6, Ach-5 and nopaline for C58), facilitated the isolation of transfer constitutive (Tra^c) Ti plasmid mutants. These studies also demonstrated that conjugation and opine catabolism are coordinately regulated in *Agrobacterium* (Klapwijk *et al.*, 1978). Agrobacteria "colonize" plants by inducing tumors to synthesize opines, which in turn provide a selective stimulation for bacteria living in the tumor to utilize the opines as sole carbon and nitrogen source, as well as to maintain the virulence by inducing the conjugation of Ti plasmids to avirulent bacteria. This mechanism was formulated as the concept of "genetic colonization" (Schell *et al.*, 1979) and as the "opine" concept by J. Tempé (Tempé and Schell, 1977; Tempé *et al.*, 1979).

The application of transposon insertion and site-specific mutagenesis techniques in conjunction with transcript mapping and sequencing studies led us and others to precise mapping, mutagenesis and functional analysis of T-DNA encoded genes. In one of the pioneering experiments, my colleague and former student, J.-P. Hernalsteens, introduced a *Tn7* transposon into a gene located close to the right boundary of the T-region of a nopaline Ti plasmid. Tumor tissues induced with the *Agrobacterium* strain carrying this construct failed to synthesize nopaline, confirming that the insertion was located in the nopaline synthase gene (*nos*) carried by the T-DNA. On the other hand, mapping of the T-DNA in transformed plants confirmed the presence of this large *Tn7* transposon insert within the T-DNA transferred to crown gall cells demonstrating that the T-DNA of Ti plasmid can be used as a versatile tool to transform plant cells with foreign DNA (Hernalsteens *et al.*, 1980). This was the real beginning for us of the era of genetic engineering of plants.

The Use of T-DNA as Plant Transformation Vector

In 1978, I (J. Schell) was invited to be the director of the Max-Planck Institute for Plant Breeding in Cologne, Germany and encouraged to develop a new project based on the use of T-DNA in plant transformation and genetic engineering. As in Gent, I also found enthusiastic collaborators in Cologne, including the biochemists L. Willmitzer, J. Schröder and L. Otten; cell biologists H.-H. Steinbiβ, O. Schieder, and newly arriving post-docs, C. Shaw, C. Koncz, D. Llewellyn, B. Baker and later many others. In addition, I received support not only from the Max-Planck Society, but also from the Genetics Institute of the University of Cologne, in particular from P. Starlinger, a well-known expert in plant transposon genetics. I was also able to maintain a strong bond and collaboration with the group in Gent until the late 1980s. Thus, we set up a very productive team to develop T-DNA as a tool for the study of molecular aspects of plant biology.

Functional analysis of the T-DNA encoded oncogenes and their protein products provided us with the possibility of studying the molecular action of plant hormones auxin and cytokinin, as it allowed us to show that the T-DNA encodes the genes for enzymes involved in the synthesis of these hormones. Inactivation of the *IaaH* and *IaaM* genes required for auxin synthesis was observed to decrease the auxin to cytokinin ratio leading to teratoma shoot formation. In contrast, abolishing the function of the *ipt* gene resulted in high auxin to cytokinin ratio promoting root formation. In addition to the functional analysis of T-DNA genes, which has been extensively reviewed (see e.g., Nester and Kosuge, 1981; Bevan and Chilton, 1982; Nester *et al.*, 1984; Morris, 1986; Binns and Thomashow, 1988; Zambryski *et al.*, 1989), we could devise various approaches to study cell differentiation and organ development, and use these tools to attempt the regeneration of fertile transformed plants. Braun and Wood (1976) claimed that teratoma shoots regenerate to fertile plants by suppression of the neoplastic state, and these results were supported by data of Sacristan and Melchers (1977) who achieved regeneration of plants from *Agrobacterium*-transformed single cell clones. However, repetition of these experiments showed that plants regenerated from teratoma shoots contained no T-DNA (see e.g., Yang *et al.*, 1980). These negative results led to the conclusion that "T-DNA cannot pass meiosis." Using a "brute force" approach, we assayed a large number of shoots regenerated from a teratoma which was induced with a T-DNA construct carrying inactivated *IaaM* and *IaaH* genes. We found, to our surprise, fertile plants which produced octopine and inherited this character in a Mendelian fashion (Otten *et al.*, 1981). The molecular analysis of these plants revealed that except for the octopine synthase gene, all other T-DNA encoded genes were deleted in these first transgenic plants. Thus, we learnt the lesson that by removal or inactivation of the T-DNA encoded oncogenes, T-DNA transformed plants can easily be obtained (De Greve *et al.*, 1982). Based on the observation that the Ti plasmid T37 incites shooting teratomas, showing very low activity of *IaaM* and *IaaH* genes, Barton *et al.* (1983) inserted a yeast alcohol dehydrogenase gene in the *ipt* gene inactivating cytokinin production. Their effort resulted in a transformed plant carrying a full-length T-DNA with the *ADH* gene, confirming the conclusion that T-DNA can successfully be used as a plant transformation vector if its oncogenes are removed and the T-DNAs are thus "disarmed."

To facilitate the introduction of foreign DNA sequences into the T-DNA, we built a commonly used cloning vector, pBR322, into the nopaline synthase gene. Foreign genes cloned in pBR322, were introduced into *Agrobacterium* by pBR322 mobilization and, because this vector was unable to replicate in *Agrobacterium*, could be stabilized by homologous recombination with pBR322 sequences within the T-DNA (Koncz *et al.*, 1984). To construct a disarmed vector, pGV3850, all DNA sequences between the T-DNA border repeats were replaced by pBR322 (Zambryski *et al.*, 1983). To select for T-DNA transformed cells, however, suitable plant selectable marker genes had to be constructed. Our experiments showed that bacterial, yeast and animal genes were either not expressed, or not properly expressed, in plants. L. Herrera-Estrella (a Mexican student) and A. Depicker used the promoter and polyadenylation signal sequences of the nopaline synthase gene and linked them to bacterial antibiotic resistance

genes to construct chimeric selectable marker genes, and introduced them into tobacco using the pGV3850 vector. Following a simple selection for antibiotic resistance, they could regenerate fully normal and fertile transgenic plants from transformed tobacco calli (Herrera-Estrella *et al.*, 1983a, b). These experiments enabled wide-range application of T-DNA as a plant transformation vector.

The Birth and Growth of Plant Molecular Biology

In the early 1980s, many of us working in tough competition on *Agrobacterium*, the Ti plasmid and T-DNA realized that the real potential for further development lays in the application of these tools to explore the function and regulation of plant genes involved in metabolism, differentiation and development. Our experience with *Agrobacterium* could be immediately applied to the study of mechanisms by which plants recognize and mount a defense against pathogens. The construction of chimeric genes and the study of their expression in plants provided a tool to examine *cis*-regulatory DNA sequences of promoters controlling the expression of plant genes in response to developmental, hormonal and environmental stimuli, as well as the nature of regulatory factors binding to these sequences. It was also clear that these approaches would never reach their goals without agricultural application, and industrial and political interest. The potential of using transgenic plants as a suitable replacement for herbicides, insecticides, fungicides, and the use of them for environmentally friendly raw material production was recognized by plant breeders and industry. This was reflected in the publication of an overview on *Agrobacterium*-mediated plant transformation and its further applications in the 1987 *Annual Review of Plant Physiology* by a leading industrial laboratory (Klee *et al.*, 1987). Concerning the recent fate of *Agrobacterium* and Ti plasmid research, we wish to emphasize only a few developments which we think merit highlighting.

Among those who devoted their research activity to clarifying the mechanisms by which *Agrobacterium* recognizes plant cells and transfers its T-DNA into the plant cell nucleus, E. W. Nester and P. Zambryski and their students and collaborators have made outstanding contributions throughout the last decade. The biology of *Agrobacterium*-mediated gene transfer has been worked out in fine detail, with the exception of the T-DNA integration mechanism which still awaits the identification of plant nuclear factors mediating this process (for review see Zambryski 1988, 1989, 1992; Lynn and Chang, 1990; Citovsky and Zambryski, 1993; Lanka and Wilkins, 1995). T-DNA vectors and *Agrobacterium* helper strains are available for plant

laboratories all over the world. Simplification of the use of T-DNA vectors goes back to the recognition that the T-DNA does not carry any gene necessary for its transfer to the plant nucleus. For DNA transfer, it is sufficient to insert genes between the two border repeats of the T-DNA which can be maintained in a plasmid replicon separated from the Ti plasmid providing the virulence (*vir*) gene functions in *Agrobacterium*. (Hoekema *et al.*, 1983). There is a wealth of different T-DNA vectors which can be simply used for cloning in such a binary vector system in order to study the activity of plant promoters with different reporter genes, or to construct chimeric genes for engineering new traits in plants, or to use antisense and overexpression approaches for inhibition or suppression of a particular gene activity by transformation. (for review see e.g., Weising *et al.*, 1988; Mazur and Falco, 1989; Klee and Estelle, 1991; Schell, 1992; Walden *et al.*, 1997).

The Use of T-DNA in Plant Genetics

Since 1983, plant molecular biology has gradually overtaken various fields of plant biology. Today, the analysis of plant gene functions, physiological and developmental processes, and many other aspects of plant biology cannot be meaningfully performed without the use of transgenic plants. Although several other transformation technologies, such as PEG-mediated direct DNA uptake into protoplasts and biolistic methods, are also available, an overwhelming majority of transformed plants are generated with T-DNA as gene vector (Birch, 1997).

In addition to the major impact of T-DNA transformation in the analysis of regulation of plant gene expression and engineering of plants with agriculturally useful properties, the use of T-DNA has contributed to substantial achievements in plant genetics. It was earlier realized that T-DNA integration into plant chromosomal DNA could occur within genes and thus cause gene mutations. To test the efficiency of T-DNA as an insertional mutagen, we used a gene fusion technology by linking promoterless reporter genes to the T-DNA end, such that T-DNA insertions in plant genes could easily be identified either by selecting or screening for the activation of reporter genes. Measurement of the frequency of T-DNA-induced gene fusions in various plants showed that 20% to 30% of all T-DNA inserts were located in genes (Koncz *et al.*, 1989)! T-DNAs carrying known DNA sequences, plant marker genes and, optionally, bacterial plasmid replicons could thus be used as efficient insertional mutagens for gene tagging, allowing simple isolation and characterization of mutant plant genes. As first examples of the application of this genetic technique, we

reported on the T-DNA tagging of the *CH42* gene (Koncz *et al.*, 1990), whereas others found T-DNA inserts in the *GL1* and *AG1* loci of *Arabidopsis* (Marks and Feldmann, 1989; Yanofski *et al.*, 1990). In our laboratory, B. Baker was the first to use T-DNA to deliver the maize *Ac* and *Ds* transposons into tobacco and to develop this method to a highly efficient insertional mutagenesis system, later together with others (Baker *et al.*, 1986, 1987).

Based on the pioneering work of G. P. Rédei (1970, 1974), *Arabidospsis*, a species with the smallest known genome among angiosperms and a short generation time, became the model system in plant biology during the late 1980s (Koncz *et al.*, 1992a; Meyerowitz and Somerville, 1994). Today, thousands of T-DNA- and transposon-tagged genes are available in *Arabidopsis* and soon T-DNA tags will be isolated in each gene of this species (Koncz *et al.*, 1992b; Forsthoefel *et al.*, 1992; Bechtold *et al.*, 1993; Feldmann *et al.*, 1994). As shown recently, genes can be identified by sequencing the junctions of T-DNA inserts in the plant DNA (Mathur *et al.*, 1998). Alternatively, insertions in sequenced genes can be found by screening approaches based on the polymerase chain reaction. After sequencing over 30,000 *Arabidopsis* cDNAs, genomic research is expected to yield the complete sequence of the *Arabidopsis* genome within a few years. We who were amazed by the speed of research developments following the "green revolution," are presently faced with a new metamorphosis in plant biology termed "functional genomics," which allows sequence-based identification of plant gene mutations and subsequent analysis of plant gene functions. It is now possible for plant biologists to gain immediate information about a favorite gene just by turning on their computers; they can begin deciphering in a systematic way the protein interactions controlling such basic cellular functions as signaling pathways and cell division. Will this type of research leave space for intuition, fantasy, and creativity leading to basic discoveries as during earlier times? In our view, certainly. Nonetheless, we agree with G. P. Rédei, who taught his students an old Indian proverb concerning history: "who does not look back, can easily lose his path."

References

Baker, B., Coupland, G., Fedoroff, N., Starlinger, P. and Schell, J. (1987) Phenotypic assay for excision of the maize controlling element *Ac* in tobacco. *EMBO J.* **6:** 1547-1554.

Baker, B., Schell, J., Lörz, H. and Fedoroff, N. (1986) Transposition of the maize controlling element *activator* in tobacco. *Proc. Natl. Acad. Sci. USA* **83:** 4844-4848.

Barton, K. A., Binns, A. N., Matzke, A. J. M. and Chilton, M.-D. (1983) Regeneration of intact tobacco plants containing full length copies of genetically engineered T-DNA and transmission of T-DNA to R1 progeny. *Cell* **32:** 1033-1043.

Bearsley, R. E. (1955) Phage production by crown gall bacteria and the formation of plant tumors. *American. Naturalist.* **89:** 175.

Bechtold, N., Ellis, J. and Pelletier, G. (1993) In *planta Agrobacterium*-mediated gene transfer by infiltration of adults *Arabidopsis thaliana* plants. *C.R. Acad. Sci. [III]* **316:** 1194-1199.

Bevan, M. W. and Chilton, M.-D. (1982) T-DNA of the *Agrobacterium* Ti and Ri plasmids. *Ann. Rev. Genet.* **16:** 357-384.

Binns, A. N. and Thomashow, M. F. (1988) Cell biology of *Agrobacterium* infection and transformation of plants. *Ann. Rev. Microbiol.* **42:** 575-606.

Birch, R. G. (1997) Plant transformation: Problem and strategies for practical application. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48:** 297-326.

Bomhoff, G. H., Klapwijk, P. M., Kester, H. C. M., Schilperoort, R. A., Hernalsteens, J.-P. and Schell, J. (1976) Octopine and nopaline synthesis and breakdown genetically controlled by a plasmid of *Agrobacterium tumefaciens*. *Mol. Gen. Genet.* **145:** 177-181.

Braun A. C. (1947) Thermal studies on the factors responsible for tumor initiation in crown gall. *Am. J. Bot.* **34:** 674-677.

Braun, A. C. (1948) Studies in the origin and development of plant teratomas incited by the crown gall bacterium. *Am. J. Bot.* **25:** 5111-519.

Braun., A. C. (1950) Thermal inactivation studies on the tumor-inducing principle in crown gall. *Phytopath.* **40:** 3.

Braun, A. C. (1956) The activation of two growth-substance systems accompanying the conversion of normal to tumor cells in crown gall. *Cancer Res.* **16:** 53-56.

Braun, A. C. (1959) A demonstration of the recovery of the crown gall tumor cell with the use of complex tumors of single cell origin. *Proc. Natl. Acad. Sci. USA* **45:** 932-938.

Braun, A. C. and Naf, U. (1954) A non-auxinic growth promoting factor present in crow-gall tissue. *Proc. Soc. Exp. Biol. Med.* **86:** 212-214.

Braun, A. C. and Wood, H. N. (1976) Suppression of the neoplastic state with the acquisition of specialized function in cells, tissues, and organs of crow gall teratomas of tobacco. *Proc. Natl. Acad. Sci. USA* **73:** 496-500.

Chilton, M.-D., Currier, T. C., Farrand, S. K., Bendich, A. J., Gordon, M. P. and Nester, E. W. (1974a) *Agrobacterium tumefaciens* and PS8 bacteriophage DNA not detected in crown gall tumors. *Proc. Natl. Acad. Sci. USA* **71:** 3672-3676.

Chilton, M.-D., Drummond, M. H., Gordon, M. P., Merlo, D. J., Montoya, A. L., Sciaky, D., Nutter, R. and Nester, E. W. (1977) Stable incorporation of plasmid DNA into higher plant cells: The molecular basis of tumorogenesis. *Cell* **11:** 263-271.

Chilton, M.-D., Drummond, M. H., Merlo, D. J. and Sciaky, D. (1978a) Highly conserved DNA of Ti plasmids overlaps T-DNA maintained in plant tumors. *Nature* **275:** 147-149.

Chilton, M.-D., Farrand, S. K., Eden, F. C., Currier, T. C., Bendich, A. J., Gordon, M. P. and Nester, E. F. (1974b) Is there foreign DNA in crown gall tumor DNA? *In Modification of the Information Content of Plant Cells*, R. Markham, D.R. Davies, D. Hopwood, and R.W. Horne, eds., Elsevier, New York, pp. 297.

Chilton, M.-D., Montoya, A. L., Merlo, D. J., Drummond, M. H., Nutter, R. C., Gordon, M. P. and Nester, E. W. (1978b) Restriction endonuclease mapping of a plasmid that confers oncogenicity upon *Agrobacterium tumefaciens* strain B6-806. *Plasmid* **1:** 147-149.

Chilton, M.-D., Saiki, R. K., Yadav, N., Gordon, M. P. and Quétier, F. (1980) T-DNA from *Agrobacterium* Ti plasmid is in the nuclear DNA fraction of crown gall tumor cells. *Proc. Natl. Acad. Sci. USA* **77:** 4060-4064.

Citovsky, V. and Zambryski, P. (1993) Transport of nucleic acids through membrane channels: snaking through small holes. *Annu. Rev. Microbiol.* **47:** 167-197.

De Greve, H., Leemans, J., Hernalsteens, J.-P., Thia-Toong, L., De Beuckeleer, M., Willmitzer, L., Otten, L., Van Montagu, M. and Schell, J. (1982) Regeneration of normal and fertile plants that express octopine synthase, from tobacco crown galls after deletion of tumour-controlling functions. *Nature* **300:** 752-755.

De Vos, G., De Beuckeleer, M., Van Montau, M., and Schell, J. (1981) Restriction endonuclease mapping of the octopine tumor inducing pTiAch5 plasmid of *Agrobacterium tumefaciens*, *Plasmid* **6:** 249-253.

Depicker, A., DeWilde, M., De Vos, G., De Vos, R., Van Montagu, M. and Schell, J. (1980) Molecular cloning of the nopaline Ti plasmid pTiC58 and its use for restriction endonuclease mapping. *Plasmid* **3:** 193-211.

Depicker, A., Van Montagu, M. and Schell, J. (1978) Homologous sequences in different Ti plasmids are essential for oncogenicity. *Nature* **275:** 150-152.

Drummond, M. H., Gordon, M. P., Nester, E. W., and Chilton, M.-D. (1977) Foreign DNA of bacterial plasmid origin is transcribed in crown gall tumors. *Nature* **269:** 535-536.

Eden, F. C., Farrand, S. K., Powell, J., Benedich, A. J., Chilton, M.-D., Nester, E. W. and Gordon, M. P. (1974) Attempts to detect deoxyribonucleic acid from *Agrobacterium tumefaciens* and bacteriophage PS8 in crown gall tumors by complementary ribonucleic acid/deoxyribonucleic acid-filter hybridization. *J. Bacteriol.* **119:** 547-553.

Engler, G., Holsters, M., Van Montagu, M., Schell, J., Hernalsteens, J.-P. and Schilperoort, R. A. (1975) Agrocin 84 sensitivity: A plasmid determined property in *Agrobacterium tumefaciens*. *Mol. Gen. Genet.* **138:** 345-349.

Feldmann, K. A., Malmberg, M. and Dean, C. (1994) Mutagenesis in *Arabidopsis*. In *Arabidopsis,*. M. Meyerowitz and C. R. Somerville, eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, pp. 137-172.

Forsthoefel, N. R., Wu, Y., Schultz, B., Bennett, M. J. and Feldmann, K. A. (1992) T-DNA insertion mutagenesis in *Arabidopsis*: Prospects and perspectives. *Aust. J. Plant Physiol.* **19:** 353-366.

Genetello, C., Van Larebeke, N, Holsters, M., Depicker, A., Van Montagu, M. and Schell, J. (1977) Ti plasmids of *Agrobacterium* as conjugative plasmids. *Nature* **265:** 561-562.

Goldman, A., Thomas, D. W. and Morel, G. (1969) Sur la structure de la nopaline, métabolite anormale de certaines tumeurs de crown gall. *C. R. Acad. Sci Paris* **268:** 852-854.

Gurley, W. B., Kemp, J. D., Albert, M. J., Sutton, D. W. and Callis, J. (1979) Transcription of Ti plasmid-derived sequences in three octopine-type crown gall tumor lines. *Proc. Natl. Acad. Sci. USA* **76:** 2828-2832.

Hamilton, R. H. and Fall, M. Z. (1971) The loss of tumor-initiating ability in *A, tumefaciens* by incubation at high temperature. *Experimentia* **27:** 229-230.

Hendrickson, A. A., Baldwin, I. L. and Riker, A. J. (1934) Studies on certain physiological characters of *Phytomonas tumefaciens*, *Phytomonas rhizogenes* and *Bacillus radiobacter*. Part II. *J. Bacteriol.* **28:** 597-618.

Hernalsteens, J.-P., van Vliet, F., De Beuckeleer, M., Depicker, A., Engler, G., Lemmers, M., Holsters, M., Van Montagu, M. and Schell, J (1980) The *Agrobacterium tumefaciens* Ti plasmid as a host vector system for introducing foreign DNA in plant cells. *Nature* **287:** 654-656.

Herrera-Estrella, L., Depicker, A., Van Montagu, M. and Schell, J. (1983a) Expression of chimeric genes transferred into plant cells using a Ti plasmid-derived vector. *Nature* **303:** 209-213.

Herrera-Estrella, L., DeBlock, M., Messens, E., Hernalsteens, J.-P., Van Montagu, M. and Schell, J. (1983b) Chimeric genes as dominant selectable markers in plant cells. *EMBO J.* **2:** 987-995.

Hoekema, A., Hirsch, P. R., Hooykaas, P. J. and Schilperoort, R. A. (1983) A binary plant vector strategy based on separation of *vir* and T-region of the *Agrobacterium*. *Nature* **303:** 179-181.

Howell, S. H. (1982) Plant molecular vechicles: Potential vectors for introducing foreign DNA into plants. *Ann. Rev. Plant Physiol.* **33:** 609-650.

Kerr, A. (1969) Transfer of virulence between isolates of *Agrobacterium*. *Nature* **223:** 1175-1176.

Kerr, A. (1971) Acquisition of virulence by non-pathogenic isolates of *Agrobacterium radiobacter*. *Physiol. Plant Pathol.* **1:** 214-246.

Kerr, A. and Htay, K. (1974) Biological control of crown gall through bacteriocine production. *Physiol. Plant Pathol.* **4:** 37-42.

Kerr, A., Manigaults, P. and Tempé, J. (1977) Transfer of virulence *in vivo* and *in vitro* in *Agrobacterium*. *Nature* **265:** 560-561

Klapwijk, P. M., Scheulderman, T. and Schilperoort, R. A. (1978) Co-ordinated regulation of octopine degradation and conjugative transfer of Ti plasmids in *Agrobacterium tumefaciens*. Evidence for a common regulatory gene and separate operons. *J. Bacteriol.* **136:** 775-785.

Klee, H. and Estelle, M. (1991) Molecular genetic approaches to plant hormone biology. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42:** 529-551.

Klee, H., Horsch R. and Rogers, S. (1987) *Agrobacterium*-mediated plant transformation and its further applications to plant biology. *Ann. Rev. Plant Physiol.* **38:** 467-480.

Klein, D. T. and Klein, R. M. (1953) Transmittance of tumor-inducing ability to avirulent crown gall and related bacteria. *J. Bacteriol.* **66:** 220-228.

Koncz C, Kreutzaler F, Kálmán Z. and Schell J (1984) A simple method to transfer, integrate and study expression of foreign genes, such as chicken ovalbumin and alpha-actin in plant tumours. *EMBO J.* **3:** 1929-1937.

Koncz, C., Chua, N.-H. and Schell, J. (1992a) Methods in *Arabidopsis* Research. World Scientific, Singapore.

Koncz, C., Martini, N., Mayerhofer, R., Koncz-Kálmán, Z., Körber, H., Rédei, G. P. and Schell, J. (1989) High-frequency T-DNA-mediated gene tagging in plants. *Proc. Natl. Acad.Sci. USA* **86:** 8467-8471.

Koncz, C., Meyerhofer, R., Koncz-Kálmán, Z., Nawrath, C., Reiss, B., Rédei, G. P. and Schell, J. (1990) Isolation of a gene encoding a novel chloroplast protein by T-DNA tagging in *Arabidopsis thaliana*. *EMO J.* **9:** 1337-1346.

Koncz, C., Németh, K., Rédei, G. P. and Schell, J. (1992) T-DNA insertional mutagenesis in *Arabidopsis*. *Plant Mol. Biol.* **20:** 963-976.

Lanka, E. and Wilkins, B. M. (1995) DNA processing reactions in bacterial conjugation. *Annu. Rev. Biochem.* **64:** 141-169.

Ledoux, L., Huart, R. and Jacobs, M. (1974) DNA-mediated correction of thiaminless *Arabidopsis thaliana*. *Nature* **249:** 17-21.

Lemmers, M., De Beuckeleer, M., Holsters, M., Zambryski, P., Depicker, A., Hernalsteens, J.-P., van Montagu, M. and Schell, J. (1980) Internal organization, boundaries and integration of Ti plasmid DNA in nopaline crown gall tumours. *J. Mol. Biol.* **144:** 353-376.

Lioret, C. (1956) Sur la mise en évidence d'un acide aminé non idenfié particulier aux tissus de crown gall. *Bull. Soc. Fr. Physiol. Veg.* **2:** 76.

Locke, S. B., Riker , A. J. and Duggar, B. M. (1939) The nature of growth substance originating in crown gall tissue. *J. Agric. Res.* **300:** 535-539.

Lurquin, F. P. (1976) Integration of exogeneous DNA in plants: A hypothesis awaiting clear-cut demonstration. In *Cell Genetics of Higher Plants*, D. Dudits, G. L. Farkas and P. Maliga, eds., Akadémiai Kiadó, Budapest, pp. 77-90.

Lynn, D. G. and Chang, M. (1990) Phenolic signals in cohabitation: Implications for plant development. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41:** 497-526.

Marks, M. D. and Feldmann, K. A. (1989) Trichome development in *Arabidopsis thaliana*. I. T-DNA tagging of the *Glabrous 1* gene. *Plant Cell* **1:** 1043-1050.

Mathur, J., Szabados, L., Schaefer, S., Grunenberg, B., Lossow, A., Jonas-Straube, E., Schell, J., Koncz, C. and Koncz-Kálmán, Z. (1998) Gene identification with sequenced T-DNA tags generated by transformation of *Agrobacterium* cell suspension. *Plant J.* **13:** 707-716.

Mazur, B. J. and Falco, S. C. (1989) The development of herbicide resistant crops. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40:** 441-470.

Menagé, A. and Morel, G. (1964) Sur la présence d'octopine dans les tissu de crown gall. *C. R. Acad. Sci. Paris.* **259:** 4795-4796.

Merlo, D. J. and Kemp, J. D. (1976) Attempts to detect *Agrobacterium tumefaciens* DNA in rown gall tumor tissue. *Plant Physiol.* **58:** 100-106.

Meyerowitz, E. M. and Somerville, C. R. (1994) *Arabidopsis*, Cold Spring Harbor Laboratory Press, Cold Spring Harbour, N. Y.

Morris, R. O. (1986) Genes specifying auxin and cytokinin biosynthesis in phytopathogens. *Ann. Rev. Plant Physiol.* **37:** 509-538.

Nathans, D. and Smith, H. O. (1975) Restriction endonucleases in the analysis and restructuring of DNA molecules. *Ann. Rev. Biochem.* **44:** 273-293.

Nester, E. W. and Kosuge, T. (1981) Plasmids specifying plant hyperplasias. *Ann. Rev. Microbiol.* **35:** 531-565.

Nester, E. W., Gordon, M. P., Amasino, R. M. and Yanofsky, M. F. (1984) Crown gall: A molecular and physiological analysis. *Ann. Rev. Plant Physiol.* **35:** 387-413.

Otten, L., De Greve, H., Hernalsteens, J.-P., Van Montagu, M., Schieder, O., Straub, J. and Schell, J. (1981) Mendelian transmission of genes introduced into plants by the Ti plasmid of *Agrobacterium tumefaciens*. *Mol. Gen. Genet.* **183:** 209-213.

Parsons, C. L. and Beardsley, R. E. (1968) Bacteriophage activity of crown gall tissue. *J. Virol.* **2:** 651.

Petit, A., Delhaye, S., Tempé, J. and Morel, G. (1970) Reserches sur les guanidines des tissus de crown gall. Mise en évidence d'une relation biochemique spécifique entre les souches d'*Agrobacterium tumefaciens* et les tumeurs qu'elles induisent. *Physiol. Veg.* **8:** 205-213.

Rédei, G. P. (1970) *Arabidopsis thaliana* (L.) Heynh. A review of genetics and biology. *Bibliogr. Genet.* **20:** 1-150.

Rédei, G. P. (1974) *Arabidopsis* as genetoc tool. *Annu. Rev. Genet.* **9:** 111-127.

Rédei, G. P., Acedo, G., Weingarten, H. and Kiehr, L. D. (1976) Has DNA corrected genetically thiaminless mutants of *Arabidopsis*? In *Cell Genetics of Higher Plants*, D. Dudits, G. L. Farkas and P. Maliga, eds., Akadémiai Kiadó, Budapest, pp. 91-94.

Riker, A. J. (1926) Studies on the influence of some environmental factors on the development of crown gall. *J. Agric. Res.* **26:** 425-435.

Sacristan, M.-D. and Melchers, G. (1977) Regeneration of plants from "habituated" and "*Agrobacterium*-transformed" single-cell clones of tobacco. *Mol. Gen. Genet.* **152:** 111-117.

Schell, J. (1975) The role of plasmids in crown gall formation by *A. tumefaciens*. In *Genetic manupulations with Plant Materials*, L. Ledoux, ed., Plenum Press, New York, pp. 163-181.

Schell, J. (1992) Plant biotechnology: A powerful tool to use plant resources and to improve the environmental impact of agriculture. In *Natural Resources and Human Health*, S. Baba, ed., Elsevier, New York, pp. 49-60.

Schell, J., Van Montagu, M., De Beuckeleer, M., De Block, M., Depicker, A., De Wilde, A., Engler, G., Genetello, C., Hernalsteens, J.-P., Holsters, M., Seurinck, J., Silva, A., Van Vliet, F. and Villarroel, R. (1979) Interactions and DNA transfer between *Agrobacterium tumefaciens*, the Ti plasmid and the plant host. *Proc. R. Soc. Lond.* **B204:** 251-266.

Schilperoort, R. A., Dons, J. J. M. and Ras, H. (1974) Characterization of the complex formed between PS8 cRNA and DNA isolated from A6-induced sterile crown gall tissues. In *Modification of the Information Content of Plant Cells*, R. Markham, D. R. Davies, D. Hopwood and R. Horne, eds., Elsevier, New York, pp.253-268.

Sinsheimer, R. L. (1977) Recombinant DNA. *Ann. Rev. Biochem.* **46:** 415-438.

Smith, E. F. and Townsend, C. O. (1907) A plant tumor of bacterial origin. *Science* **25:** 671-673.

Southern, E. (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98:** 503-517.

Tempé, J. and Schell, J. (1977) Is crown gall a natural instance of gene transfer? In *Translation of Natural and Synthetic Polynucleotides*, A. B. Legocki, ed., University of Agriculture, Poznan, pp. 416-420.

Tempé, J., Guyon, P., Tepfer, D. and Petit, A. (1979) The role of opines in the ecology of the Ti plasmids of *Agrobacterium*. In *Plasmids of Medical, Environmental, and Commercial Importance*, K. Timmis and A. Pühler, eds., Elsevier, Amsterdam, pp. 353-363.

Thomashow, M. F., Nutter, R., Montoya, A., Gordon, M. P. and Nester, E. W. (1980) Integration and organization of Ti plasmid sequences in crown gall tumors. *Cell* **19:** 729-739.

Van Larebeke, N., Engler, G., Holsters, M., Van den Elsacker, S. Zaenen, I., Schilperoort, R. A., and Schell, J. (1974) Large plasmid in *Agrobacterium tumefaciens* essential for crown gall-inducing ability. *Nature* **252:** 169-170.

Van Larebeke, N., Genetello, C., Schell, J., Schilperoort, R. A., Hermans, A. K., Hernalsteens, J.-P. and Van Monagu, M. (1975) Acquisition of tumor-inducing ability by non-oncogenic agrobacteria as a result of plasmid transfer. *Nature* **255:** 742-743.

Walden, R., Reiss, B., Koncz, C. and Schell, J. (1997) The impact of Ti plasmid-derived gene vectors on the study of the mechanism of action of phytohormones. *Annu. Rev. Phytophatol.* **35:** 45-66.

Watson, B., Currier, T. C., Gordon, M. P., Chilton, M.-D. and Nester, E. W. (1975) Plasmid required for virulence of *Agrobacterium tumefaciens*. *J. Bacteriol.* **123:** 255-264.

Weising, K., Schell, J. and Kahl, G. (1988) Foreign genes in plants: Transfer, structure, expression, and applications. *Annu. Rev. Genet.* **22:** 412-477.

Willmitzer, L., De Beuckeleer, M., Lemmers, M., Van Montagu, M. and Schell, J. (1980) The Ti plasmid derived T-DNA is present in the nucleus and absent from plastids of plant crown gall cells. *Nature* **287:** 359-361.

Willmitzer, L., Otten, L., Simons, G., Schmalenbach, W., Schröder, J., Schröder, G., Van Montagu, M. and Schell, J. (1981) Nuclear and polysomal transcripts of T-DNA in octopine crown gall suspension and callus cultures. *Mol. Gen. Genet.* **182:** 255-262.

Yadav, N. S., Postle, K., Saiki, R. K., Thomashow, M. F. and Chilton, M.-D. (1980) T-DNA of a crown gall teratoma is covalently joined to host plant DNA. *Nature* **287:** 458-461.

Yang, F.-M., Montoya, A. L., Merlo, D. J., Drummond, M. H., Chilton, M.-D., Nester, E. W. and Gordon, M. P. (1980) Foreign DNA sequences in crown gall teratomas and their fate during the loss of the tumor traits. *Mol. Gen. Genet.* **177:** 704-714.

Yanosfski, M. F., Ma, H., Bowman, J. L., Drews, G. N., Feldmann, K. A. and Meyerowitz, E. M. (1990) The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. *Nature* **346:** 35-38.

Zaenen, I., Van Larebeke, N., Teuchy, H., Van Montagu, M. and Schell, J. (1974) Supercoiled circular DNA in crown gall inducing *Agrobacterium* strains. *J. Mol. Biol.* **86:** 109-127.

Zambryski, P. (1988) Basic process underlying *Agrobacterium*-mediated DNA transfer to plant cells. *Annu. Rev. Genet.* **22:** 1-30.

Zambryski, P. C. (1992) Chronicles from the *Agrobacterium*-plant cell DNA transfer story. *Annu. Rev. Plant Physiol.* **43:** 465-490.

Zambryski, P., Holsters, M., Kruger, K., Depicker, A., Schell, J., Van Montagu, M. and Goodman, H. M. (1980) Tumor DNA structure in plant cells transformed by *A. tumefaciens*. *Science* **209:** 1385-1391.

Zambryski, P., Joos, H., Genetello, C., Leemans, J., Van Montagu, M. and Schell, J. (1983) Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity. *EMBO J.* **2:** 2143-2150.

Zambryski, P., Tempé, J. and Schell, J. (1989) Transfer and function of T-DNA genes from *Agrobacterium* Ti and Ri plasmids in plants. *Cell* **56:** 193-201.