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Chilton M-D, Drummond M H, Merlo D J, Sclaky D, Montoya A L, Gordon M P & Nester E W. Stable incorporation of plasmid DNA into higher plant cells: the molecular basis of crown gall tumorigenesis. *Cell* 11:263-71, 1977.

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The plant tumor called crown gall grows cancerously because of new genes inserted into its chromosomes by the pathogenic bacterium, *Agrobacterium tumefaciens*. The transferred DNA is part of a virulence plasmid carried by the bacterium [The SCJ® indicates that this paper has been cited in over 335 publications since 1977]

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Crown gall plant tumors, caused by the common soil bacterium *Agrobacterium tumefaciens*,<sup>1</sup> were known to contain plant cells that seemed genetically different from normal ones. These cells, when grown in a culture free from bacteria, would seemingly "remember" orders dictated by the inciting bacteria.<sup>2</sup> Many researchers in the field believed there must be gene transfer from the pathogen to the plant cell. In the 1960s and early 1970s, numerous attempts were made, using filter-bound DNA hybridization, to find bacterial DNA in tumor DNA, with controversial and irreproducible results.

In 1974 Jeff Schell's group at the University of Ghent, Belgium, discovered that virulent *Agrobacteria* contained large plasmids.<sup>3</sup> These subsequently were proved to be required for tumor formation and were named Ti (tumor-inducing) plasmids. If gene transfer were the basis of the disease, it seemed plausible that the transferred genes should come from the Ti plasmid.

Our group at the University of Washington used DNA renaturation kinetics to look for Ti plasmid sequences in DNA isolated from plant tumor cells. The experiment consisted of mixing tumor DNA or normal (control) DNA with labeled Ti plasmid DNA and then measuring the rate of renaturation of the labeled DNA. If the tumor DNA contained copies of sequences found in the plasmid, the tumor DNA would make the plasmid DNA probe renature faster. Initial-

ly, we concluded that the whole plasmid certainly was not present. To test for the presence of individual parts of the plasmid, we labeled Ti plasmid *in vitro* with <sup>32</sup>P, cut it with the restriction endonuclease Sma I, and, by agarose gel electrophoresis, separated 17 fragments that were eluted and used in kinetic studies.

Logistically, the *in vitro* labeling of the DNA had to be started on Thursday, the day that freshly prepared labeled nucleotide arrived at the lab. By Friday afternoon the probes were ready and we prepared 85 different DNA mixtures, each of which had to be divided and sealed in nine capillary tubes, one for each kinetic point. The start of the experiment was almost a ballet. Every 20 seconds a set reaction was placed in a 105° C bath to denature for exactly five minutes, then placed in the 63° C renaturation bath. Exactly five minutes later, the proper reaction was located and a capillary was removed for the first kinetic point. As timekeeper, Milt Gordon stood over us with the stopwatch calling, "Ten seconds.... Go!" for each maneuver. Seven of us continued to work around the clock from Friday evening until Sunday morning taking time points and processing samples to collect the data before <sup>32</sup>P decay damaged the DNA irretrievably.

Our four-day "brute force" experiments showed that fragment 3b of the Ti plasmid was in the tumor DNA. We now call the part of the plasmid transferred to the plant cell T-DNA. The discovery of T-DNA was a shock to many biologists in an era of controversy over the safety of recombinant DNA experiments: here was a microorganism operating outside the NIH guidelines, joining its plasmid DNA to higher plant DNA. Our paper was so controversial that we were required by the referees to provide additional experimental evidence before it was finally accepted for publication.

T-DNA transfer is now exploited by genetic engineers for introduction of desirable genes into plant cells that can regenerate into plants.<sup>4</sup> Our publication has been cited frequently because it provided the key evidence for a biological process of fundamental interest to geneticists, of utility to plant molecular biologists, and of great potential value to plant genetic engineers.

1 Smith E F & Townsend C O. A plant tumor of bacterial origin. *Science* 25 671-3, 1907 (Cited 90 times since 1955)

2 Petit A, Delhaye S, Tempé J & Morel G. Recherches sur les guandines des tussus de crown gall. Mise en évidence d'une relation biochimique spécifique entre les souches d'*Agrobacterium tumefaciens* et les tumeurs qu'elles induisent. *Physiol Vég* 8 205-13, 1970. (Cited 170 times)

3 Zaenen I, van Larebeke N, Teuchy H, van Montagu M & Schell J. Supercoiled circular DNA in crown-gall inducing *Agrobacterium* strains. *J Mol Biol* 86 109-27, 1974 (Cited 265 times)

4 Chilton M-D. A vector for introducing new genes into plants. *Sci Amer* 248 50-9, 1983