Homology between Ti-Plasmids of *Agrobacterium tumefaciens*:
Hybridization Studies using Electron Microscopy

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Crown-gall is a neoplastic disease that can affect all dicotyledonous plants (Braun, 1947). The agent inducing this disease is a Gram-negative bacterium, *Agrobacterium tumefaciens*.

All oncogenic *Agrobacterium* strains contain at least one large plasmid (Zaenen et al., 1974) called Ti-plasmid. Curing of the plasmid abolishes oncogenicity. Reintroduction of a Ti-plasmid, by transformation, co-transfer or conjugation, re-establishes the tumour-inducing property (Van Larebeke et al., 1975). Genetic and 'restriction-enzyme fingerprint' analysis (Schell et al., 1976; Depicker et al., 1977), and *Cot* hybridization (Currier & Nester, 1976) indicate that most of the Ti-plasmids studied until now belong to two general types. One represents a group of highly related plasmids isolated from octopine-utilizing *Agrobacterium* strains called Ti-ocu plasmids; the other type represents the plasmids isolated from nopaline-utilizing *Agrobacterium* strains called Ti-nou plasmids.

The electron microscope was used to study the sequence relation between different Ti-plasmids isolated from *Agrobacterium tumefaciens*. Hybridization conditions were as described by Sharp et al. (1972), except for a few modifications. Owing to the 63% G+C content of the plasmids, it was necessary to heat the DNA to 50–55°C, during alkaline denaturation, to achieve complete strand dissociation. To avoid nicking, the DNA was not concentrated by precipitation and the hybridizations were done at relatively dilute DNA concentrations. Nicking could not be avoided, so relatively few complete circular heteroduplexes were observed. Most measurements were carried out on heteroduplex structures formed between plasmid fragments.

Hybridizations between plasmids, isolated from two octopine strains *A. tumefaciens* B6S3 and *A. tumefaciens* ACH5, never showed regions of non-homology, so that we can conclude that there is a 100% sequence homology. To be sure that we were not dealing with homoduplexes, a co-integrate plasmid between the Ti-plasmid of *A. tumefaciens* B6S3 and the P-type plasmid RP4 was used instead of the *A. tumefaciens* B6S3 Ti-plasmid (Genetello et al., 1977).

When hybridizations were carried out between plasmids isolated from different nopaline-utilizing *Agrobacterium* strains, such as *A. tumefaciens* Kerr 14 and *A. tumefaciens* C58, different short regions of non-homology with lengths ranging from 0.1 to 2 µm were observed. These stretches of non-homology were distributed over the entire plasmid genome; many of them appeared as 'eye-like' structures, suggesting that inversions of DNA sequences have occurred during plasmid evolution in *Agrobacterium*.

Heteroduplex molecules between Ti-ocu and Ti-nou plasmids were not previously found. In this experiment we used plasmid DNA from *A. tumefaciens* C58, a nopaline-utilizing strain, and *A. tumefaciens* B6S3, an octopine-utilizing strain. The reason for this failure is probably due to the fact that there is not enough homology between these
Comparative Study of Ti-Plasmids in Agrobacterium tumefaciens by Use of Restriction Enzymes

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That the plant-oncogenic properties of Agrobacterium tumefaciens are related to the presence in these strains of large plasmids (the Ti-plasmids) has been demonstrated in a number of ways.

1) Curing of the Ti-plasmid results in the irreversible loss of oncogenicity (Engler et al., 1975).

2) Transfer of Ti-plasmids to non-oncogenic plasmid-less strains confers oncogenicity to these strains (Genetello et al., 1977).

Since the contour length of the Ti-plasmids varies between 50 and 70 μm and since some phenotypic properties of identical bacteria differ when they carry different Ti-plasmids, it was obvious that more than one type of Ti-plasmid existed. However, since all of these different Ti-plasmids can cause tumour formation, it could be assumed that they all should have a common sequence.

Our purpose was a comparative study between Ti-plasmids from different oncogenic Agrobacterium strains by means of "fingerprints" of restriction-nuclease digests (Sugden et al., 1975). From these results, we characterized at least two groups: (1) the Ti-plasmids which carry the genes for octopine catabolism (Ti-ocu plasmids) have very similar restriction patterns; (2) the Ti-plasmids coding for the nopaline-catabolizing enzymes (Ti-nou plasmids) show a larger differentiation in the restriction "fingerprints", but they can clearly be recognized as a group. The most important result was the very low similarity between restriction patterns of ocu and nou Ti-plasmid DNA.

We determined the homology between the two groups by hybridizing a radioactive reference Ti-plasmid against ocu and nou plasmid DNA digests, separated on gel and transferred to a nitrocellulose filter (Southern, 1975). With the restriction enzyme EcoRI, we found only two such fragments with the same electrophoretic mobility, common to nine different Ti-plasmids and hybridizing equally well to ocu and nou