

The Plasmids of Agropine-Type *Agrobacterium rhizogenes* Strains: Physical and Preliminary Functional Maps

A.M. BIROT, D. BOUCHEZ, F. CASSE-DELBART, L. JOUANIN, J. TOURNEUR and F. VILAINE

Laboratoire de Biologie Cellulaire, CNRA, Route de Saint Cyr, F78000 Versailles, France

Tel. (1)30.21.74.22 Telex INBAUER 695269F

Abstract

The restriction maps of the plasmids of *A. rhizogenes* strains HRI and A4 were established and compared. The respective ability of the TL-DNA and the TR-DNA alone to induce root proliferation was compared on various plant species. Homologies with pTi fragments containing individual defined *vir* genes were localized on the virulence region of pRiA4. The replication origins of pARA4a and pRiHRI were localized precisely and used to construct shuttle cosmids.

Keywords: plasmid, *Agrobacterium*

Two regions of wild-type agropine Ri plasmids can be transferred to the plant genome: the TL-region shares homology with the single T-region of mannopine Ri plasmids, while the TR-region possesses homology with the pTi T-DNA genes responsible for auxin (tms) and agropine (ags) synthesis. Deletions generated *in vitro*, as well as cloning of each T-region in a wide host range cosmid made it possible to construct strains which possess only one of the two T-regions (Fig. 2). These strains were used to compare the respective ability of the TL-DNA and the TR-DNA alone to induce root proliferation on various plant species. Strains possessing only the TL-DNA induce proliferation of more transformed roots than those possessing the TR-DNA alone when inoculated on explants of tobacco, endive, potato, carrot and sugar beet; in contrast, the TR-DNA appears more efficient than TL-DNA on explants of pea and tomato (Vilaine and Casse-Delbart, in press).

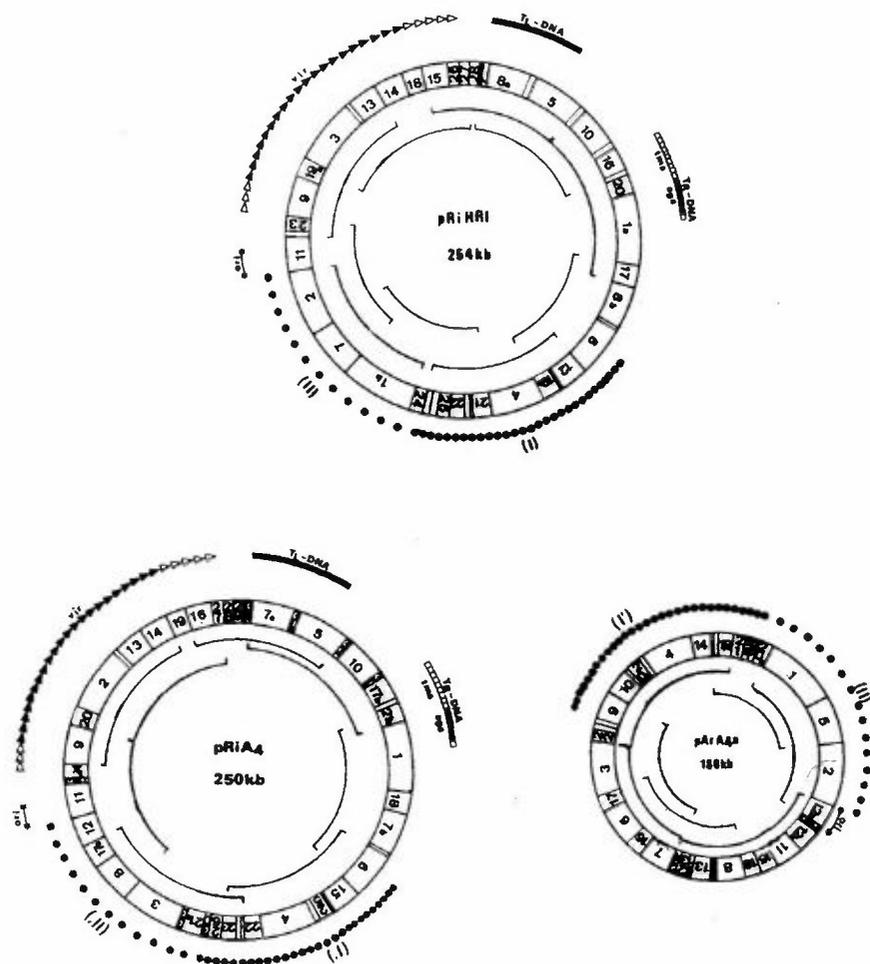


Figure 1. Bam HI maps of the plasmid of strain HRI (pRiHRI) and the plasmids Ri and a of strain A4 (pRiA4 and pArA4a). In the interior of the maps the inserts of some chosen hybrid cosmids are represented. On the exterior, the conserved regions (I and I'), the modified regions (II and II'), the *vir* region, the T-regions and the origins of replication are indicated.

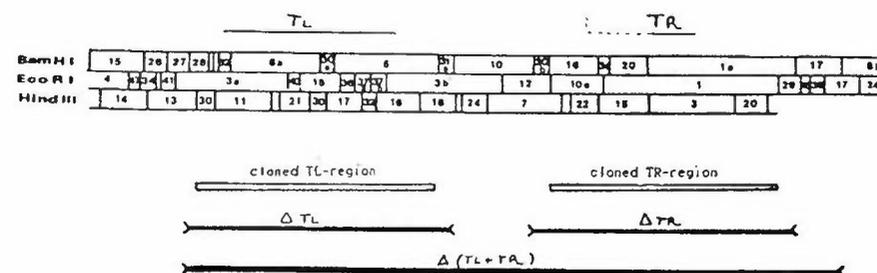


Figure 2. Under the restriction map of the T-region of pRiA4 are indicated the extent of the fragments cloned in the wide host range cosmid pLAFRi (open bars) and the size of the deletions generated in pRiA4 (black bars).

The virulence region of pRiA4 was studied by hybridization with pTi fragments containing individual defined *vir* genes. Localization of homologies revealed that regions equivalent to *vir* A, B, C, and D of pTi Ach5 (Klee et al., 1983) are more closely linked on pRiA4 than on pTiAch5; only faint homology was observed with the fragment containing *vir* E locus, and no homology with *vir* F.

The replication origins of pArA4a and pRiHRI were localized precisely and used to construct shuttle cosmids able to replicate both in *E. coli* and *Agrobacterium* where they are stably maintained even without selection pressure (Jouanin et al., 1985).

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