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## T-DNA

Much of plant genetics and crop improvement involves transformation — moving foreign DNA into a plant. The foreign DNA can be from another organism or from the same organism in a rearranged form.

### Agrobacterium tumefaciens

Crown gall; a plant 'cancer'. Agrobacterium genetically transforms the cell.

You can kill the bacterium and the gall still grows

Bacteria-free galls grow in culture without added hormones

The galls produce unusual amino acids called opines, which the plant can't use, but the bacterium can.

Agrobacterium contains a large (250Kb) plasmid, known as the Ti (tumor-inducing) plasmid. Different types of Ti exist and are named with respect to which opines they regulate e.g. octopines or nopalines. The bacterium infects the gall but only outside of the cells.

### Parts of the Ti plasmid

Three important regions

#### vir

Necessary for gall formation

8 operons (virA through vir H) with 24 genes that code for transfer functions

Thought to have evolved from bacterial conjugation process

#### T-DNA

Actually transferred into the plant cell:

Cytokinin gene - isopentyl transferase makes a cytokinin (which is not usually used by the plant)

Two auxin biosynthetic genes: tryptophan indole acetamide IAA. The first step, catalyzed by AUX1 is a monooxygenase and the second step catalyzed by AUX2 is a hydrolase.

Opine metabolism gene.

#### opine catabolism

genes necessary for bacterium to use opines as a carbon and nitrogen source

Can mutate these genes: mutate either aux gene, get shooty phenotype; mutate the cytokinin gene, get rooty phenotype; mutate the opine biosynthetic gene, bacteria can't live on the gall.

### The transfer process

1) Plant is wounded.

During the repair process, the plant secretes acetosyringone and other phenolics used in cell wall synthesis, as well as sugars & low pH.

2) VirA product is in the cell wall of the bacterium, and recognizes acetosyringone, passes the information (phosphate group) onto virG, which activates the entire vir region. Bacteria attach to cell walls.

3) T-DNA is mobilized. VirD2 attaches to the Right Border (RB; 5' end) and initiates a strand-displacement reaction whereby a single strand of the T-DNA is removed. This is then bound by VirE2 and the strand is moved into the plant cell. Products of the virB operon are used in the transfer process. Potential mechanisms are:

bacterial cell conjugates with the plant cell

bacterial cells within wounded cells die, releasing strand which then goes into live cells by plasmodesmata

VirE2 is like a viral protein and the DNA is injected that way.

4) T-DNA integrates into the plant genome 3' end first through a region of microhomology. The region of homology can be well past the LB and even in the gene(s) you wish to transfer. For this reason, the selectable marker (kan) is always in the 3' end.

### **Ti Plasmid is engineerable**

Can remove of the T-DNA region for the Ti plasmid to create a "binary" system. T-DNA can be mobilized in *trans*. The big plasmid is called the disarmed Ti-plasmid and the small one the binary vector.

Can then remove ALL of the inside portions of the TDNA (except left and right border) and replace with whatever you want. This usually involves three things: antibiotic resistance for propagating in bacteria (e.g. tetracycline, not within TDNA borders) kanamycin resistance and an MCS (which are within the borders). Can also have a reporter gene within the borders such as green fluorescent protein (screening for GFP ensures that the kanamycin-resistant cells have not escaped from selection). Can put a lot in there because TDNA can transfer >100kb.

A gene of interest is inserted into the MCS. The gene must have a promoter such as CAMV (cauliflower mosaic virus) and a polyadenylation site.

-DNA often inserts in multiple copies.

### **Host range**

TDNA can be used to transform a wide variety of dicots: Arabidopsis, potato, tomato, walnut, alfalfa, tobacco, cotton, peanut, soybean, lettuce, etc.

It has been possible to transform monocots such as maize by constitutively expressing the vir region, or by adding second copies of only three vir genes: vir B (pore in bact. wall), vir C (host range) and vir G (activator of all vir genes).

### **Uses**

Making new mutants (approach pioneered by Ken Feldmann)

Simple binary vector with antibiotic resistance

Immerse plant in solution containing Agrobacterium. Grow out seeds and a ~1% of the seeds have received the T-DNA.

All new mutations are heterozygous, implying that infection does not happen until right around meiosis or fertilization

### **Uses (cont'd)**

Verifying a cloned gene by complementing the mutant phenotype.

Crop improvement

### **Other methods of gene transfer**

Electroporation. An electric discharge make holes in an a protoplast's membrane so that DNA can enter the cell. This is generally used only for transient assays.

Biolistic transformation (microprojectile bombardment; gene gun). Very easy in rice. The basic idea is to extract immature embryos, blast tissue with DNA-coated tungsten or gold particles, and regenerate plants under selection. In maize, roughly 1 transformant per 100 immature embryos bombarded.