Crown gall disease is a neoplastic growth (i.e. "cancer") affecting many types of plants, primarily woody dicots and gymnosperms, but sometimes even mosses. The disease is caused by infection by Agrobacterium tumefaciens strains carrying a Ti (tumor inducing) plasmid. A similar disease called "hairy root" is caused by infection by A. rhizogenes strains carrying related plasmids.

Reasons for studying crown gall disease:

• economic importance in agriculture
• only known system involving effective cross-domain DNA transfer
• model for neoplastic transformation
• model system for plant-microbe interactions (some similarity to Rhizobium nodulation)
• Ti plasmid as rDNA vector in genetic engineering of crop plants

General properties of the genus Agrobacterium:

• Gram-negative; alpha-Proteobacteria; related to Rhizobium, several genera of phototrophs, and to mitochondrial ancestor.
• rods
• flagellated
• obligate aerobes with respiratory metabolism
• metabolize glucose via Entner-Doudoroff Pathway
THE SEQUENCE OF MOLECULAR INTERACTIONS BETWEEN HOST PLANT, BACTERIUM AND PLASMID LEADING TO CROWN GALL FORMATION IS COMPLEX:

1. **WOUNDING**

   Gall formation can be initiated on any aerial portion of the plant. However, galls are most frequently observed at the root crowns, close to the soil where *A. tumefasciens* is likely to be present and because cultivated plants are prone to wounding in this region by mechanical soil tillage practices.

2. **CHEMOSENSORY SIGNALING**

   Certain "phenolics" are excreted at high levels by plants in response to wounding. (Ironically these have been considered to be "defense compounds"). The phenolics are chemoattractants for *Agrobacterium* and induce expression of *vir* genes of the Ti Plasmid.

3. **RECOGNITION and BINDING**

   Plant Pectin – Bacterial Beta-glucans in Outer Membrane

   *A. tumefasciens* binds specifically to plant cells exposed in wounds. Binding requires *Agrobacterium* chromosomal genes *chvA*, *chvB*, and *exoC*, which are constitutively expressed and required for synthesis and export of a cyclic Beta-1,2 glucan exopolysaccharide and cellulose. Analogous genes are required for nodulation of legumes by *Rhizobium*.

4. **VIR GENE EXPRESSION**

   Plant phenolics which induce *vir* gene expression: include acetosyringone, catechol, p-OH-benzoic acid, pyrogallic acid and vanilli
virA  • environmental sensor
   • integral membrane protein with sensory domain exposed to periplasmic space where it binds plant phenolics;
   • catalytic domain (activated by phenolic binding to sensory domain) exposed to cytoplasm where it activates the VIR G protein by phosphorylation
   • Vir A is autophosphorylated at a his residue (histidine protein kinase)

virG  • positive regulatory protein for transcription of other vir transcriptional units;
   • phosphorylated at asp^{52} by VIR A

The virA and virG gene products comprise a typical 2-component signalling system reminiscent of MCP + CheA.

virB  • transfer of T DNA; membrane protein; pilus assembly, “conjugation bridge”

virC  • transfer of T DNA

virD  • transfer of T DNA; T-DNA border endonuclease

virE  • transfer of T DNA
   • ss DNA binding protein (Science, 240, 501.)

5. T DNA TRANSFER

The 25 kb segment of the Ti plasmid is transferred as a ss DNA fragment from bacterial cell to plant cell. Transfer mechanism may be similar, to some extent, to DNA transfer during plasmid mediated conjugation. Note, however, that DNA transfer to plant is directed by expression of vir genes not tra genes.
6. ds DNA SYNTHESIS AND INTEGRATION OF T DNA IN HOST PLANT GENOME

T DNA is stably incorporated in host plant nuclear DNA and may be transmitted through the germ line. There are many potential sites of integration in the plant genome, preference for inverted or direct tandem repeats. Expression of T DNA genes can be influenced by their location.

7. TUMOR INDUCTION

Can be directed entirely by onc genes (tms1, tms2, tmr, ipt) of T DNA integrated in the host plant genome without further participation of A. tumefaciens.

onc genes code for enzymes which synthesize plant hormones that stimulate plant cell proliferation. These genes have standard expression signals (5’ TATA boxes, 5’ CAAT boxes and 3’ AATAAA boxes) appropriate for eukaryotic genes (no introns) and are transcribed by the plant cell RNA polymerase II.

**Monoxygenase pathway for synthesis of auxin**

Also: Isopentyl transferase (ipt) converts AMP to isopentenyl AMP, a compound with cytokinin activity.
8. **OPINE SYNTHESIS AND EXCRETION FROM TRANSFORMED PLANT CELLS**

In addition to the tumor-inducing genes, the T DNA expresses genes (ocs, etc.) for one or several novel amino acid derivatives known collectively as opines.

![Opine Synthesis by Reductive Condensation of an α-Keto Acid with an Amino Acid](image)

Different strains of Ti plasmid will synthesize different opines by using different combinations of alpha-keto acid and amino acid.

9. **OPINE UTILIZATION**

*cat* genes on Ti plasmid code for opine-induced transport (permease) and catabolism (oxidase) as source of C, N and energy for the *Agrobacterium*. *cat* gene products are specific for cognate opine. Opines also induce conjugal transfer of Ti plasmid to *Agrobacterium* cells lacking plasmid.

**BIOLOGICAL CONTROL OF CROWN GALL DISEASE BY A. radiobacter**

*A. radiobacter* is a close relative of *A. tumefaciens*. It does not harbor a Ti plasmid and it cannot induce gall formation. It does however invade and inhabit crown galls. It produces an antibiotic, *Agrocin 84*, that is a fraudulent adenine nucleotide. The *A. tumefaciens* octopine permease transports Agrocin 84 into the cell where it inhibits DNA synthesis.

Cultures of *A radiobacter* are a very successful biological control agent. Seeds or seedlings are exposed to the culture before planting to prevent crown gall formation.
REFERENCES

Winans (1992)
Two-Way Chemical Signaling in Agrobacterium-Plant Interactions.
Microbiological Reviews 56:12.

DISCUSSION QUESTION

Agrobacterium cells can be eliminated from crown gall tumors by treatment with antibiotics. Yet, the tumors continue to grow after the bacteria have been eradicated. How can this be?