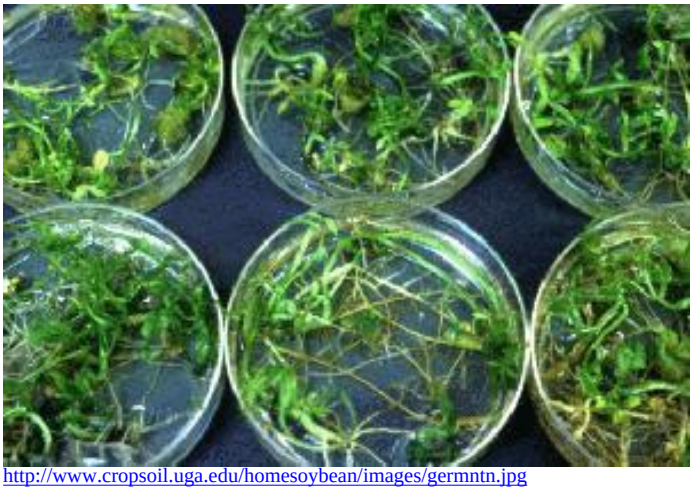
Lecture 20

Transgenic plants

Plant cells are totipotent, and this sets them apart from animals!

Plant cloning Plants can be cultured in the laboratory in nutritive agar, regenerated from single cells.



http://www.cropsoil.uga.edu/homesoybean/images/germntn.jpg

Genetic modifications can be made in somatic cells, and the modified somatic cell can develop into an adult plant that is of single-cell origin.

That's a pretty recent advance in genetics, of course. One of the best examples of the gradual domestication of plants through genetics is that of corn, as described by Buckler in the Genetics of Maize Domestication. The food purists will want to take note - every ear of corn is genetically modified, and has been for thousands of years.

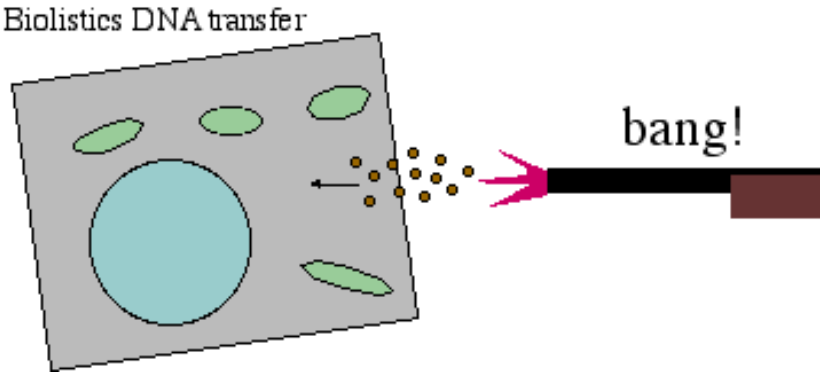
Introduction of DNA into plant cells

Well, one problem with working with plants is that they have a cell wall - how do you get a recombinant plasmid to cross that barrier? Some strategies are microinjection of single cells, electroporation of cells grown without a cell wall (protoplasts), biolistics, and Agrobacterium-mediated

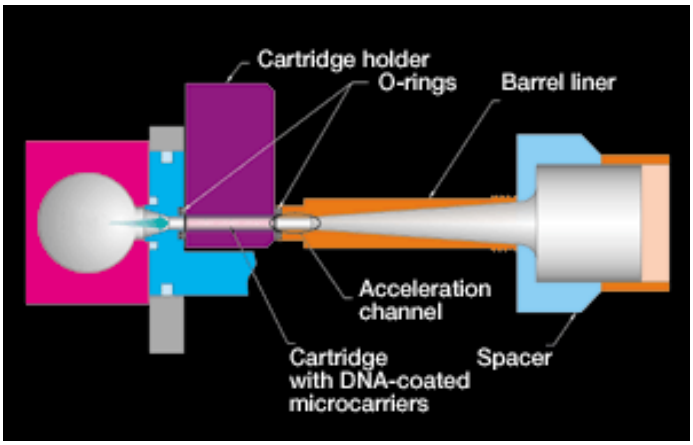
transfer.

One very common way of introducing DNA into plant cells is through DNA coated particles (e.g. gold 1 micron particles) that are literally shot through the cell wall.

This is sometimes called "biolistics" - a cross between biology and ballistics. Another term for the device is a "gene gun"



How does it work? There are a variety of different engineering strategies, but one way is to accelerate the particles using a pulse of helium.



http://www.Bio-Rad.com/images/gene_gun_delivery.gif

For example, here's a model you can get from BioRad, for example. It's probably best not to have one of these sitting on the front seat of your car if you get pulled over by the police.

Biorad's Helios Gene Gun

Picture source: http://www.bio.davidson.edu/courses/Bio111/gun.gif

Agrobacterium - mediated transfer

Here's an entirely different approach - one that harnesses a natural transfer system in many types of plants. Plants develop crown galls upon infection with *Agrobacterium*, and this involves the transfer of genes from the bacterium to the plant.

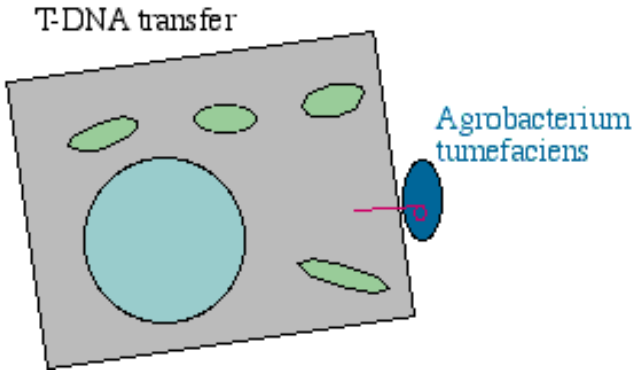
Pathogenesis of Crown Gall (shockwave page)-Sforza et al.

- Schematic distribution of crown gall pathogen and vectors
- Rough draft of Ti plasmid

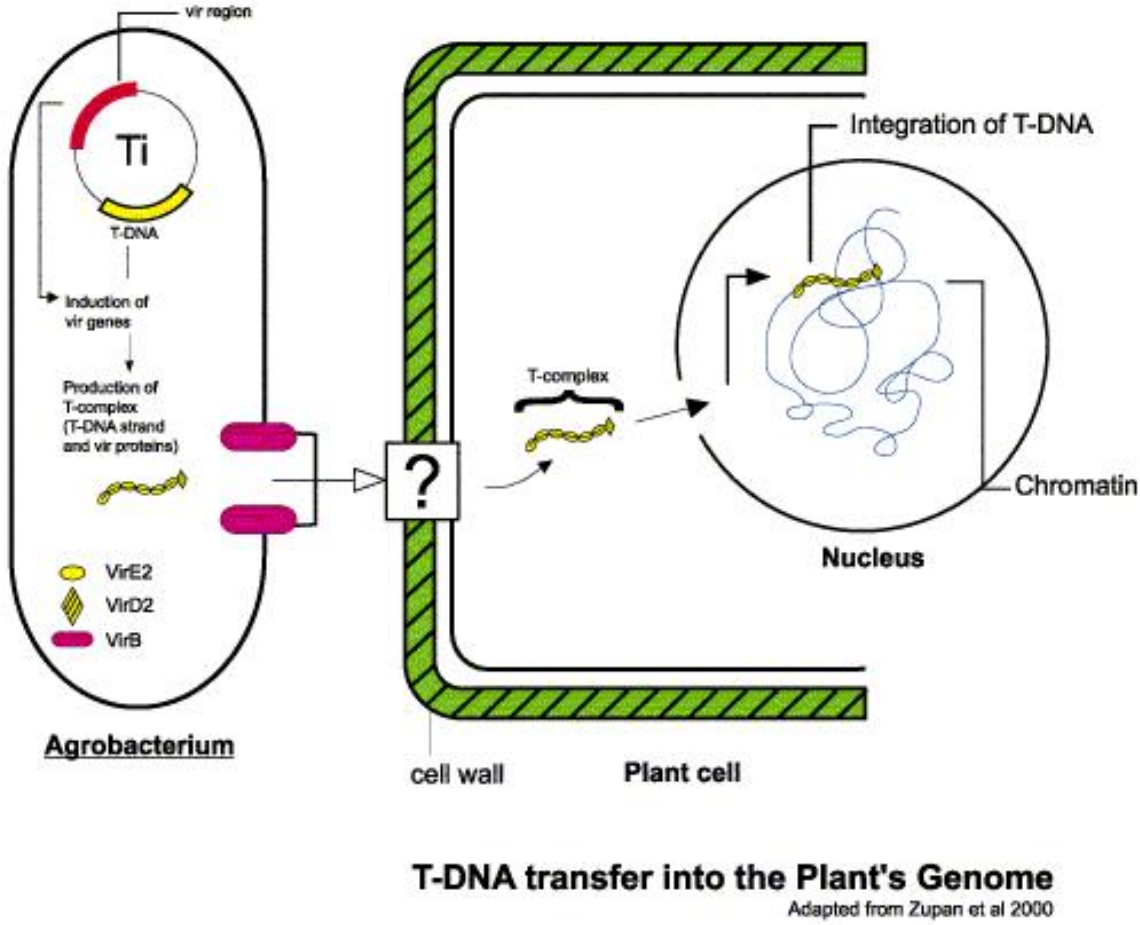
Additional resource readings:

Agrobacterium - the microbe of the month - microbes.org
Bacterial Crown Gall of Fruit Crops - Ohio State University

The method of transfer is not unlike bacterial conjugation:



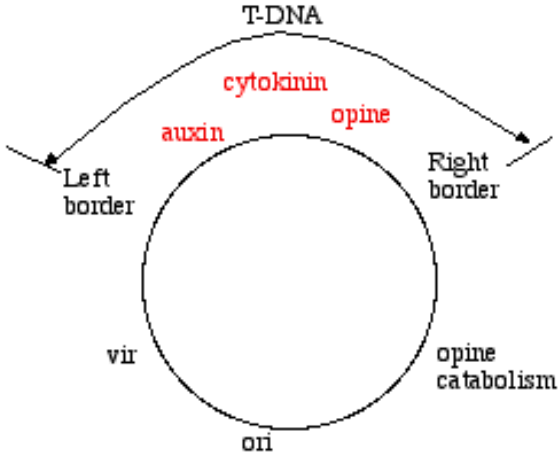
Here's a schematic diagram (from cambiaip.org) that is detailed, although the exact mechanism of transfer is highlighted by the question mark:



Picture source: http://www.cambiaip.org/cambiaIP/diags/transfer_1.jpg

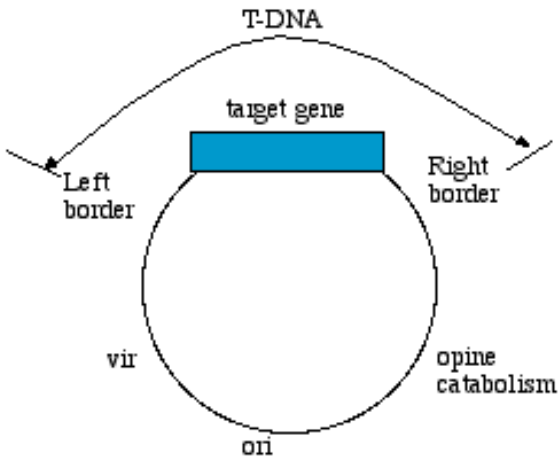
Resource Reading: From CAMBIA Intellectual Property Resource, a 262 page "white paper" with detailed information on Agrobacterium transformation, intellectual property, and patents: *(pdf format)*

Agrobacterium tumefaciens carries a plasmid (Ti) with a number of important features:

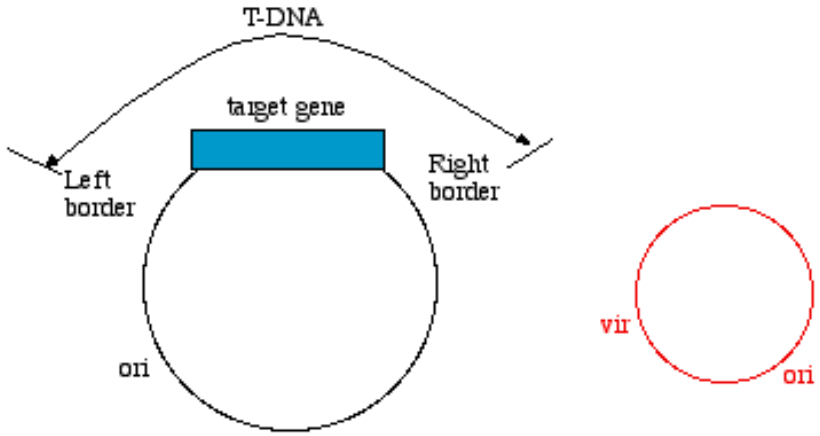


The "vir" or virulence functions are trans-acting elements that mobilize a plasmid containing the right border element (a cis-acting element). The transfered DNA is the region between the right and left border sequences, and includes genes that are tumorigenic (auxin and cytokinin production), and a gene the directs synthesis of specific opines (sugar derivatives that are not easily catabolized by other species). The genes for opine catabolism stay with the specific A. tumefaciens species, allowing that bacterium to benefit from the opine production of the plant.

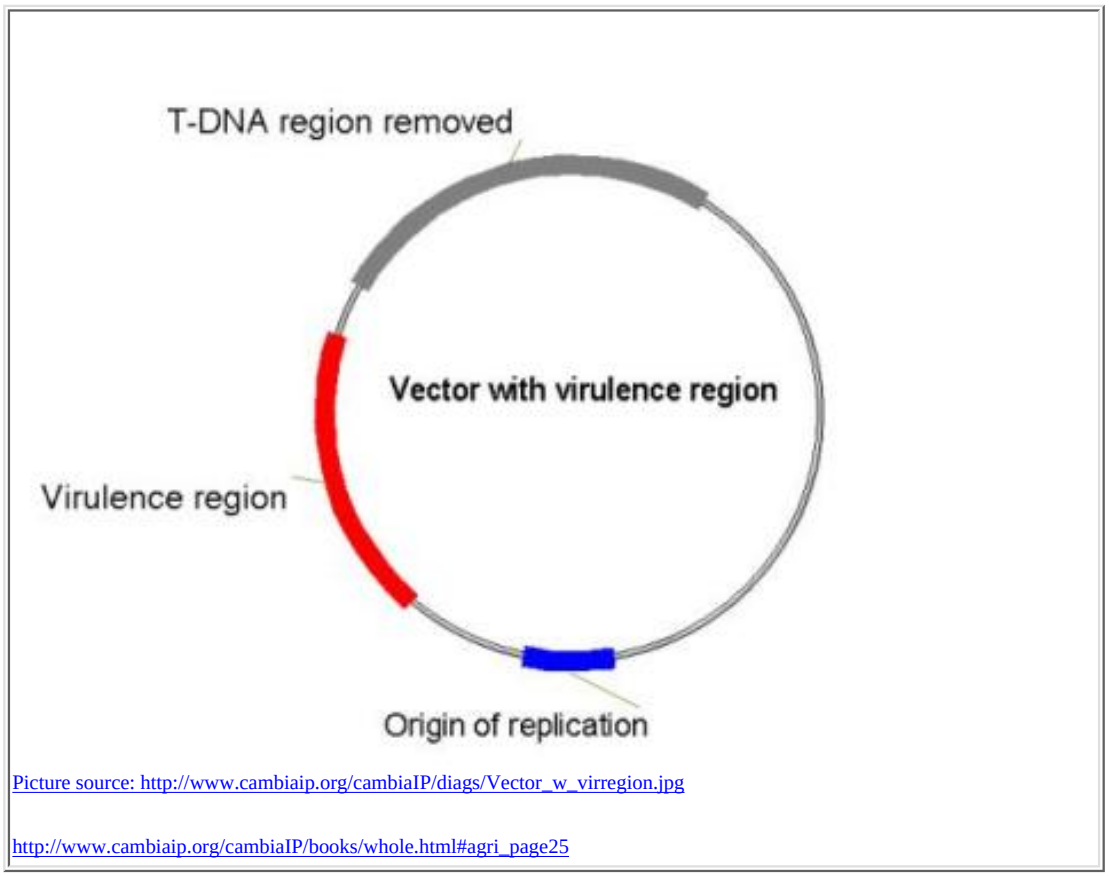
Engineered Ti vectors may simply delete the tumorigenic and opine-specific genes:



Or alternatively, the virulence genes may be moved to a different non-mobilized vector (since they are active in trans)



Binary vector systems are widely used, and here's what a "helper" vector might provide:



Picture source: http://www.cambiaip.org/cambiaIP/diags/Vector_w_virregion.jpg

http://www.cambiaip.org/cambiaIP/books/whole.html#agri_page25

If the "helper" has the vir genes, then what does the second plasmid carry? A right border sequence (and perhaps a left border sequence as well), a promoter and gene of interest, and selectable markers for selection in *E. coli*, *A. tumifaciens*, and plants.

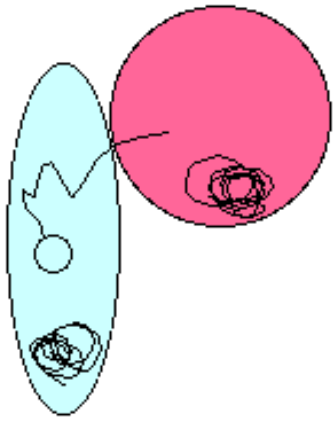
Now hold that thought! We need to think some more about the underlying principles.

cis and trans revisited What is the difference between an element required in cis, and one required in trans?

> ...a trans element can be moved to a different location, for example from the Ti plasmid to the genome, and it will still function effectively. A gene that makes a diffusible protein product can be moved generally, and that is an example of a "trans" element. An example of a "cis" element is an origin of replication. That cannot be moved, and still serve its purpose. It must be "on" the DNA that needs replicating.

Moving day! Now how do we move a big plasmid from *E. coli* to *A. tumifaciens*?

Bacteria do not need to be the same species to conjugate, and so it is possible to transfer large segments of DNA.



While there is some variability in the effectiveness of E. coli regulatory sequences in other species of bacteria (and vice versa), it is often possible to get a single gene or operon to perform well in two different hosts. Plasmids can participate in conjugation (like an F factor) if they contain the "mobilization" functions that allow them to replicate as a rolling circle and enter the conjugation bridge. The "conjugation" functions (the genes responsible for the connection between the bacteria) are different, and may or may not be encoded on the plasmid.

For example, the F factor we have discussed has both conjugation and mobilization functions, because it carries the trans-acting genes that make the bacteria male, and yet also the cis-acting DNA elements that lead to plasmid transfer.

In fact, we are not limited to just moving genes from E. coli. We can get other bacterial species involved in sharing their genes efficiently, but it takes a bit of work. Here's an example of tripartite mating:
I guess this is what is commonly referred to as a "threesome". Three

bacterial strains are mixed.

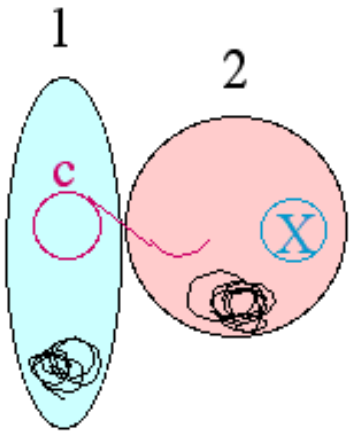
Bacteria 1: Is unable to grow in nutrient deficient media, and carries a plasmid with conjugation and mobilization functions. The bacteria are sensitive to an antibiotic (call it "X")

Bacteria 2: Is also unable to grow in nutrient deficient media, does not have conjugation functions but does have a mobilizable plasmid carrying a gene providing resistance to antibiotic X, as well as a gene of interest to us that we would like to move to "Bacteria 3".

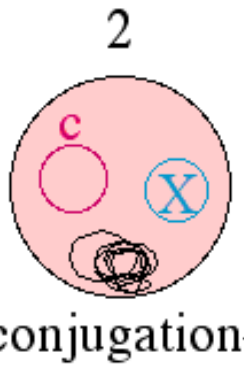
Bacteria 3: Carries no plasmid, and can grow on nutrient-deficient ("minimal") media, and is sensitive to antibiotic X.

Mix them together, and:

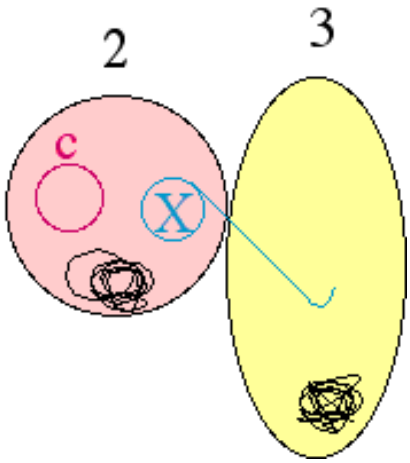
- Bacteria 1 conjugates with Bacteria 2, making it conjugation-positive by transfer of the plasmid.



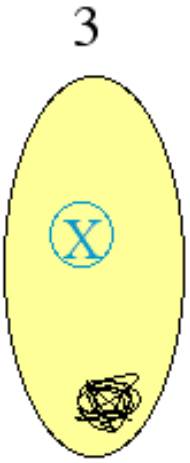
This is what you get...



- Bacteria 2 may now conjugate with Bacteria 3, and transfer the "X" resistance plasmid, which contains our gene of interest.



And this is what you get in the end:



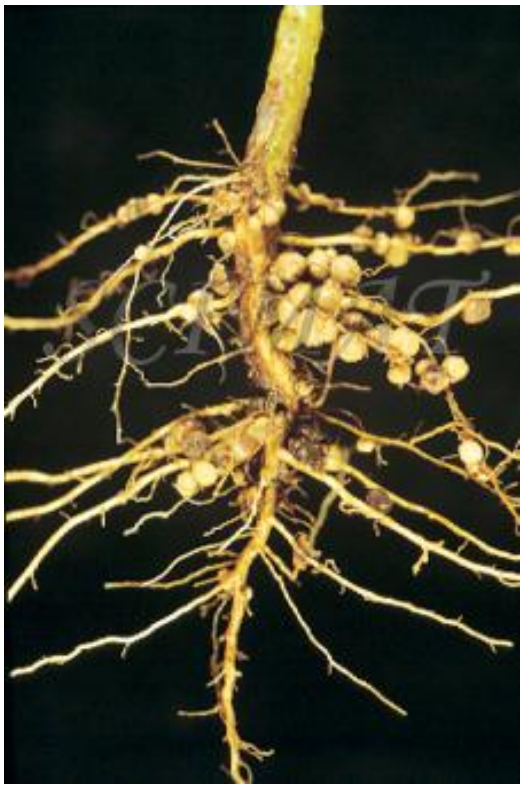
grows on minimal media
plus antibiotic X

The net result is that you were able to transfer a DNA of interest (associated with antibiotic resistance marker X) into a new species of bacteria.

With mobilization functions on a PAC or a BAC, it is possible to move large (100-250 kbp) pieces of DNA, and this allows entire operons to be moved. Now imagine the possiblities!

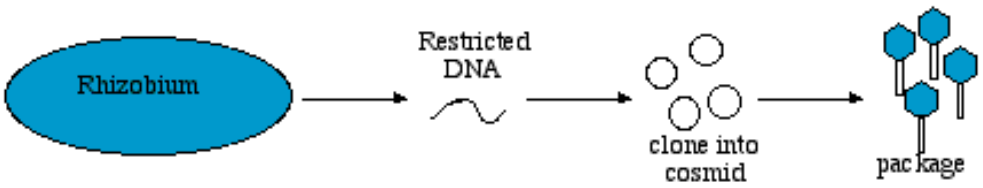
Suppose you want to find all the bacterial genes involved in root nodulation in a nitrogen fixing bacterium

Nodules full of nitrogen-fixing bacteria on the roots of a soya plant

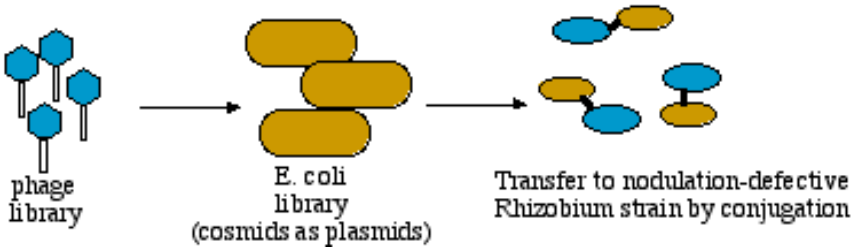


Picture Source: http://distans.livstek.lth.se:2080/rootnodules.htm

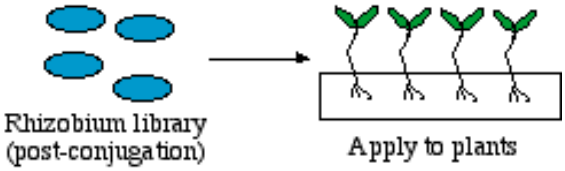
This is called cloning by genetic complementation, and it goes something like this. Start with a nodulating bactererium, such as Rhizobium meliloti, and isolate genomic DNA. Digest the DNA with a restriction enzyme and make a cosmid library.



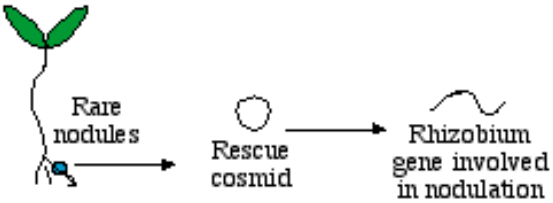
Now if you infect E. coli with the cosmid library, you essentially have an E. coli plasmid library (remember that the cosmids grow as plasmids, not as phage). Using the method of conjugation, you can transfer the library into a nodulation-defective strain of Rhizobium.



Wow! Now you've got a Rhizobium library, and if you apply it to plants, you will get an occasional nodule forming.

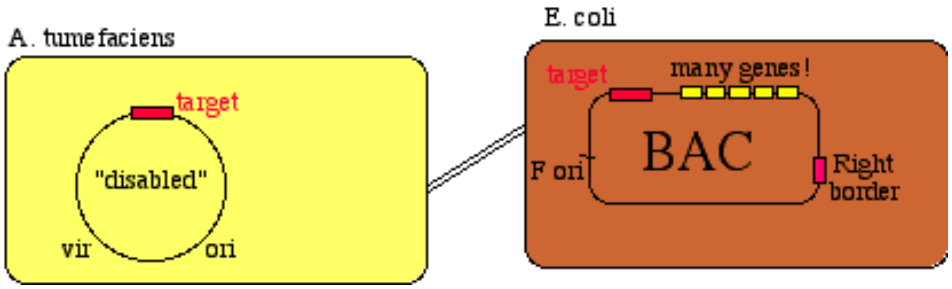


What do these rare nodules represent? They represent complementation of the nodulation-defective phenotype by a particular plasmid in the library. That plasmid is likely to carry a piece of Rhizobium DNA that corrects the defect in the nodulation-defective strain of Rhizobium.



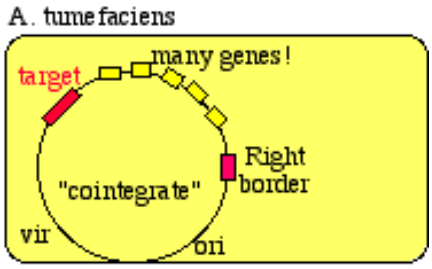
Back to the farm Now let's get back to the real subject at hand, which was all of the methods of introducing DNA into plant cells.

Bacterial origins of replication are species specific, so you could start with a disarmed Ti plasmid in Agrobacterium (disarmed in the sense that it is missing the cis-elements necessary for T-DNA mobilization). The disabled vector would be replication competent, and would carry a target sequence for recombination. This strain of Agrobacterium could be conjugated to E. coli carrying a bacterial artificial chromosome (BAC) or even a BAC library of plant DNA. The E. coli plasmid does not have an origin of replication that would function in A. tumefaciens, so it can only survive by integration into the disabled A. tumefaciens plasmid. This would all be handled by a selectable marker (not shown):



After recombination between the similar target sequences (which could be

mediated by cre-lox if you want to get fancy) the A. tumefaciens strain would bear a cointegrate vector that carries both the virulence genes and the cis-acting border sequence:



Now, this can be applied to plants, and the T-DNA element containing the BAC genes can be transferred into plants.

We have moved genes from one plant species to another.

Bt crops Here is an important example of the use of biotechnology in plant crops.

Bacillus thuringiensis has been used for decades as a "natural" insecticide, and is often used by organic farmers. The bacterium synthesizes a protein (a procrystal), that when consumed by larvae of certain insects is activated in the gut of the insect, and becomes a pore in the membrane of the intestinal epithelium. The bacterial crystals are biodegradable (in sunlight) and are not hazardous to humans. In Bt crops, the same protein is expressed within the tissues of the plant, so application of the bacteria or the purified crystal is not necessary.

Constitutive promoters lead to expression of Bt protoxin in all tissues, including root and pollen. This is both an advantage (in range of effectiveness) and a disadvantage (in rate of biodegradation)

Risks and benefits Ecological concerns include increased risk of invasiveness and volunteerism of the crop, hybridization (intra and interspecies), effects on non-target organisms and management of resistance. There are also concerns about product toxicity and allergenicity.

Volunteerism

Some of last year's crop may grow alongside the current crop

Invasiveness

Strengthening "weed-like" crops like canola, sunflower and rice may not be such a good idea. Soybean and corn are not able to prosper outside of agricultural setting, so that may be less of a concern.

Intraspecific hybridization

Saving seed from prior years' harvest could help modified strains persist. Wind-pollination of grains such as corn can also spread traits within a species. Organic farmers could find that their crops were being pollinated by GM pollen.

Interspecific hybridization

Some crops have weedy close relatives, and transfer of fitness-enhancing characteristics could be a problem. A weed could become more invasive and competitive, not only in the agricultural field but within the natural ecosystem. For example, alfalfa and rice have close relatives in the wild. The domestic and wild species must share some sexual compatibility and have sufficient chromosome homology so that a viable hybrid can be formed.

An herbicide-resistant weed would not be predicted to have much of a gain in fitness in the natural ecosystem. On the other hand, an insect-resistant weed could have a significant increase in fitness, because there are plenty of insect pests in the wild.

Keep in mind that hybridization that has been typically used in agriculture (for hundreds or thousands of years) leads to the transfer of thousands of genes from one plant into another, often across species lines. How do you estimate the risk of that "reckless" practice with the intentional transfer of a

single gene by recombinant methods?

Effects on non-target organisms

...on insect predators:

Lacewings (Chrystoperla carnea) that were allowed to feed on European corn borers (Ostrinia nubilalis) reared on Bt corn had a higher mortality than those feeding on control corn borers

Monarch butterfly larvae (Danaus plexippus) were allowed to consume milkweed leaves that had be dusted with Bt corn pollen, and had decreased survival rates. This was meant to model feeding of larvae on milkweed near Bt cornfields, but it should be noted that the levels of pollen in the study may have been artificially high. Other studies have shown that Bt corn has no effect on swallowtail butterflies.

Allergic reaction to foods

It is difficult to determine what causes food allergies, but transgenic crops do have the potential of causing problems for individuals. For example, a transgenic soybean expressing a Brazil nut albumin (to increase methionine content of the crop) was found to be recognized as an antigen by sera from individuals allergic to Brazil nuts. This line of soybean was not commercialized, but if it had been one can imagine that soybeans would become hazardous to people allergic to Brazil nuts (soybeans from different sources are all mixed together by distributors, so all soybean products could potentially contain this foreign protein).

Benefits

Application of chemicals

Bt crops result in fewer treatments with broad-spectrum insecticides (a cited example was that Bt cotton required three insecticide treatments per year compared with 5-12 insecticide sprays in non-transgenic cotton fields. It is estimated that use of Bt cotton may have reduced pesticide use by over 900,000 kg during 1997. Decreased pesticide use certainly changes the business of farming (i.e. the profit structure) and may be viewed as a benefit to the environment.

Assessment of risk

Approximately 75 million acres of transgenic crops are grown in the U.S., amounting to approximately 2.5 trillion transgenic plants during the past dozen years. There is little evidence to suggest that this has caused any hazard to human health or the environment. We are not turning into Teenage Mutant Ninja Turtles.

http://www.ninjaturtles.com/logos/logoanim.gif

Feeding the world and improving the diet of people.

Approximately 800,000,000 people are in areas of the world that are "food insecure", most of these being in Asia and Africa. A quarter of this at-risk poulation are children. Vitamin A deficiency is a critical problem that causes eye damage to an estimate 14 million children under age 5. About one billion people are at risk of iron deficiency, and this is exacerbated by certain tropical diseases. This is happening during a time when global food prices are declining.

- Increasing population leads to increased demand for food, and decrease in space and available water for farming
- Increasing income leads to greater demand for animal products
- Marine fish production is not keeping pace with demand
- Ecological damage reduces arable land

One benefit to the third world would be stress-resistant crops - increasing arable lands to include fields that are poisoned by high levels of metal salts (such as aluminum).

Background reading: a Transgenic Crops - resource guide from Colorado State University

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