will attach to the DNA of the single phage that contains a copy of the element, and it will not attach to the other phage DNA's. In this manner, the d^m gene controlling plant height has been tagged with a radioactive signal and can be separated from all the other plant genes.

Once the gene that controls plant height has been isolated, it can be characterized to decipher the particular protein information it encodes and how this protein works in the plant.

Applications of Transposon Tagging

The basic approach of transposon tagging previously described is highly simplified. Although it has been used extensively in bacteria and in *Drosophila melanogaster* (fruit fly), the only plant in which the method has been used to date is *Zea mays* (corn). Some elements that inactivate genes have been identified by variegation effects or DNA sequence features that indicate transposable element action in several higher plant species.

In contrast to the wealth of knowledge about transposable elements in corn, genetic and molecular descriptions of mobile elements in other plants are much more limited. Future research will attempt to determine whether transposable elements from corn or other species will move within the chromosomes of new host plants into which they are transferred by genetic engineering rather than sexual crossing.

Plant biotechnology in the future will benefit by modification and transfer of genes for disease resistance and other valuable agronomic traits in higher plants. Mobile elements and other recently emerging approaches, such as mapping DNA by restriction fragment length polymorphisms (RFLPs) provide a way by which to identify and physically isolate these genes.

Genes for Pathogenicity

George H. Lacy, *associate professor, Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg*

Molecular techniques for studying genes for pathogenicity have provided powerful insights for understanding the complex interactions among pathogens and their plant hosts. Further, these insights have provided ideas that may be exploited for controlling plant disease.

Pathogenicity is defined here as the ability of a disease-causing organism to establish a symbiotic relationship with a host plant that includes infecting, colonizing, and damaging the host plant. Genes for pathogenicity are sequences of DNA-mediating rearrangement of pathogenic genomes and regulation or synthesis of proteins or other compounds involved in the phenotype for pathogenicity.

Infection

The infection process consists of penetration of the host by the pathogen and establishment of a nutritional relationship with the host. For penetration to occur, the bacterium must come into contact with and, in many cases, attach to the host. For example, agrobacteria attach to plant cell walls through interactions of bacterial lipopolysaccharides with plant cell wall carbohydrates. More secure attachment is provided by cellulose fibrils produced by the bacterium which lace bacterial cells to the plant cell surface. Genetic elements related

to processes for attachment and cellulose fibril formation have not been located on plasmids and will probably be found on the bacterial chromosome.

Penetration. Penetration of bacteria into their hosts occurs by several methods: they may be forced into plant tissue by water congestion, they may be introduced into wounds, they may penetrate directly, or they may be injected into their hosts by sucking insects. Molecular studies of the soil bacterium *Streptomyces scabies*, causal agent of potato scab, have led to the cloning and characterization of DNA sequences mediating an extracellular cutinase that may be involved in direct penetration of the mycelium of the pathogen through the cuticle into its host.

Agrobacteria, which cause crown gall (*Agrobacterium tumefaciens*) and hairy root (*A. rhizogenes*), are especially interesting since the oncogenicity-inducing plasmids, pTi from *A. tumefaciens* which controls tumor formation and pRi from *A. rhizogenes* which controls root formation, contain within their genomes portions of DNA that are inserted directly into the host plant's genome. The major portion of the pTi or pRi plasmids does not become incorporated and is probably degraded by host endonucleases.

Since only these oncogenicity-mediating gene sequences (TDNA's) are required for pathogenicity, they are pathogens while both the bacterium and the pTi or pRi plasmids are merely biological and molecular vectors, respectively. The processes by which the plasmids and the TDNA's penetrate through plant cell walls, cross the plasmalemma, and arrive in the nucleus have not been described; however, proteins produced from genes located on pTi are known to be involved in DNA transfer to plant cells.

Nutritional Relationship. For infection to succeed, a nutritional relationship must be established between the pathogen and its host. Detecting this relationship is understandably difficult; however, for bacteria a nutritional relationship has occurred and infection has taken place at the point when the lag phase following penetration ends and the exponential growth phase begins.

Pathogenic TDNA's pose a special problem. Like viruses, they do not have nutritional processes of their own, but depend instead upon the host's synthetic pathways to increase their numbers. In this case, a nutritional relationship has been established at the point following its recombination into the host genome and at the initiation of host directed-DNA synthesis of the TDNA. For pathogenic TDNA's, establishment of a nutritional relationship depends upon insertion of the pathogenic TDNA into the host genome by an unknown process. DNA border sequences, however, have been identified at the left and right ends of the inserted TDNA that are involved in successful recombination. Further, the sequences from the right border are absolutely required and may replace entirely the sequences for the left border for successful insertion. These border sequences fit the definition for genes for pathogenicity although no protein products have been associated with them.

Host Range

The host range of plant pathogenicity usually refers to the range of different genera and species on which a particular pathogen may cause disease. In this usage, host range represents the basic compatibility between a pathogen and its host that allows disease development. The best molecular studies of host range have been performed with agrobacteria and have

revealed that the plasmids related to oncogenicity-mediated differences in host range.

Molecular studies revealed that a portion of the pTi plasmid outside of the TDNA region is responsible for the differences in host ranges. This region of pTi has been named the virulence region, and several *vir* genes have been described within that region. It is unfortunate that the term *vir* was used to indicate genes controlling host range since it compounds the confusion created by an alternate use of the term virulence.

It has been learned, however, that exposure to plants of different species induces the formation of different proteins. This suggests strongly that host range differences require production of gene products by the pathogen. In the future, these studies will lead to a molecular knowledge of basic compatibility between pathogens and their hosts.

Overcoming Host Resistance

The term virulence also is used to mean the ability of pathogens to overcome host resistance. This usage differs from the usage concerned with host range since it refers to host-specificity among cultivars of plants within a species rather than the ability to cause disease among a group of species or genera.

It follows that pathogens are organisms capable of causing disease on some host plant. Nonpathogens, then, do not cause disease on any host plant. Virulent pathogens, however, can cause disease on a particular cultivar of a plant species, avirulent pathogens do not cause disease on a resistant cultivar, but can cause disease on some susceptible cultivar of the same species.

A virulent pathogen on its susceptible host cultivar comprises a compatible interaction. Conversely, an avirulent pathogen on a resistant host cultivar yields an incompatible interaction. Virulence is superimposed over basic host range compatibility and comprises a fertile area for molecular research into the interactions of pathogens and their hosts.

For some time it has been recognized that specific genes in the host mediate specific resistance to specific races of pathogens. Further, it has been found that the incompatible or race-specific resistance is mediated by the presence of a specific gene in the pathogen. In other words, specific incompatibility implies that a gene-for-gene relationship exists between the pathogen and its incompatible host. Avirulence genes, then, differ from host range genes in that they block pathogenesis rather than enable pathogenesis. It is possible, therefore, that virulence genes exist as separate entities from host range genes and modify the expression of host range genes in response to the presence of resistance genes in the host.

Support for this model is found in the discovery that avirulence genes may be moved from an avirulent strain of *Xanthomonas campestris* pv. *vesicatoria,* causal agent of bacteria spot of peppers and tomatoes, to a virulent strain and cause an incompatible interaction in the presence, but not the absence, of a single host resistance gene.

Other studies have indicated that transfer of an avirulence gene from one species of a pathogen to a second results in the second species developing incompatible reactions to the resistance gene specific for the first pathogen. In this case, an avirulence gene from *Pseudomonas syringae* pv. *glycinea* was transferred to *Xanthomonas campestris* pv. *glycines,* and both soybean pathogens responded in an incompatible manner to the pseudomonad resistance gene in soybean lines.

Host Damage

For pathogens, damage caused to the host is related to the production of toxins, enzymes, or plant growth regulating compounds that result in cellular disruption and release substances useful for pathogen nutrition. In soft rot pathogenesis, degradative enzymes such as pectate lyases, polygalacturonases, cellulases, proteases, and phosphokinases or phospholipases destroy elements of plant cells and release cell wall sugars, amino acids, or components of cell membranes for the use of the pathogen.

Chief among the aims of molecular studies will be the discovery of the coordination of the various enzymes required to cause soft rot and elucidation of the regulation of the enzymes. These studies will indicate how soft rot pathogens differ from several nonpathogenic organisms that have many if not all of the enzymes system apparently required for pathogenesis, yet remain saprophytes unable to colonize living plant tissue.

The production of low molecular weight toxins is an important mechanism for plant damage for several plant pathogens. In the case of phaseolotoxin produced by *Pseudomonas syringae* pv. *phaseolicola,* causal agent of halo blight of bean, molecular studies have located several genes probably in the biosynthetic pathway for toxin production. Further, molecular mechanisms for resistance to their own toxins have been discovered in *P. syringae* pv. *phaseolicola* as well as in *Pseudomonas syringae* pv. *tabaci* which produces tabtoxinine beta-lactam.

One possible outcome of this research will be the transfer of genes for these resistance factors from the pathogen for ornithine carbamoyltransferase (resistant to phaseolotoxin) or glutamate synthetase (resistant to tabtoxinin beta-lactam) into plant genomes to create crops resistant to toxin damage.

Tumor-causing bacteria such as *Agrobacterium tumefaciens* and *Pseudomonas syringae* pv. *savastanoi,* cause plant damage through alteration in levels of plant growth regulating compounds such as auxins or cytokinins. For the olive knot organism (*P. syringae* pv. *savastanoi*), two genes for auxin production are located either on a plasmid or in the chromosome of the pathogen. This pathogen modifies its host by secreting auxins into the plant tissue.

For the pathogenic TDNA of the crown gall organism, the picture is different. In this case, genes for both cytokinin and auxin production are carried on the TDNA, inserted into the host plant's genome, and expressed using eukaryotic rather than prokaryotic transcription and translation machinery. In this second model, the pathogen has changed the genetic machinery of the host to produce the plant growth regulating compounds.

Researchers have already taken advantage of the ability of the TDNA to insert into host DNA to engineer plants for resistance to antibiotics, herbicides, and disease resistance by deleting the information for the genes mediating plant growth regulating compounds and replacing them with the desired genes.