

How pests and pathogens cause plant disease

14

OM14.1 GROWTH OF *MAGNAPORTHE ORYZAE* IN RICE LEAVES

The interior of the fungal cell maintains a very high turgor (or hydrostatic) pressure. The turgor pressure is generated by the breakdown of storage compounds – lipids and a starch-like glucose polymer called glycogen – contained in the conidium, to produce glycerol. Accumulation of glycerol draws water into the fungus, raising the internal pressure against the fungal cell wall (see Section 8.1 for more explanation of turgor pressure). The cell wall of the appressorium and penetration peg can resist the high internal pressure because it is strengthened by deposition of melanin, a strong, cross-linked polymer derived from the amino acid tyrosine. Entry of the penetration peg is also assisted by the production and secretion of fungal enzymes that degrade plant cell walls in its immediate vicinity. The fungal cell wall has a different composition from the plant cell wall – it consists mostly of chitin – so production by the fungus of pectinases and cellulases that degrade plant cell walls has no effect on its own cell wall. Once inside the plant cell, the penetration peg gives rise to specialized hyphae that invade living rice cells while enclosed in a membrane derived from the host plasma membrane. As the fungus grows from cell to cell via the plasmodesmata through the leaf, a biotrophic interfacial complex (BIC) forms between the fungal and host cells from which secreted effectors from the fungus are delivered into the cytoplasm of the host cell (Figure OM14.1-1). These effectors can move ahead of the hyphae via plasmodesmata into uninfected cells, likely preparing these cells for invasion. Other fungal effectors are secreted into the apoplast between the hyphal wall and the invaginated plasma membrane of the host cell.

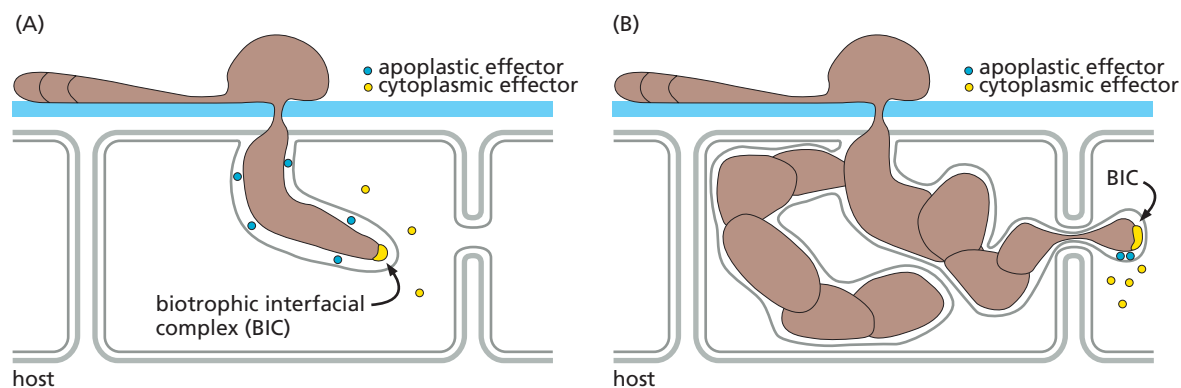


Figure OM14.1-1 *Magnaporthe oryzae* (rice blast) infection. (A) Cartoon showing a spore from which an appressorium has grown, enabling fungal entry into a leaf epidermal cell. As the hypha grows within the cell, a biotrophic interfacial complex (BIC) forms at its tip through which pathogen effectors are delivered into the plant cell. (B) As the fungus continues to proliferate within the leaf, it begins to move cell to cell via plasmodesmata, with a BIC forming at the hyphal tip in the newly colonized cell. (Continued)

(C)

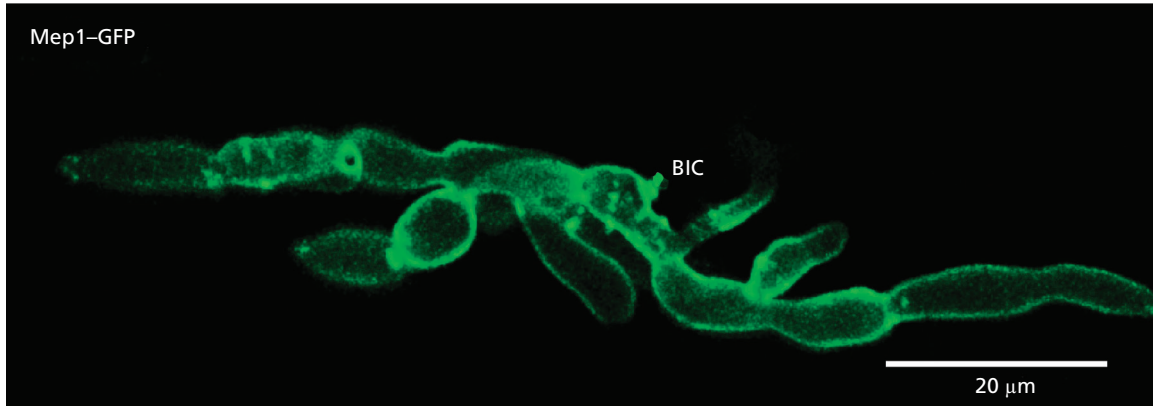


Figure OM14.1-1 (Continued) (C) Micrograph of fluorescence in a leaf colonized by a strain of *M. oryzae* expressing a fusion of an apoplastic effector gene *MEP1* to a gene encoding GFP. Mep1-GFP accumulates in the fungal apoplast between the fungus and the membrane of the plant cell, giving green fluorescence especially at BIC sites. (C, courtesy of Lauren Ryder, Neftaly Cruz-Mireles and Nick Talbot, The Sainsbury Laboratory, Norwich.)

OM14.2 QUORUM SENSING IN PLANT-PATHOGENIC BACTERIA

Bacterial quorum sensing uses mechanisms that were first discovered during studies on a symbiotic interaction between the luminescent marine bacterium *Vibrio fischeri* and marine animals including some squid and fish species. *V. fischeri* bacteria accumulate in specialized light organs in their host animals, such as the bobtail squid (**Figure OM14.2-1**). When the bacterial population reaches a sufficient density, the cells become luminescent, but quorum sensing ensures that energy is not expended in luminescence production when the density of bacteria is too low for the light organ to become visibly luminescent.

In *Erwinia carotovora*, quorum sensing enables the production of cell wall-degrading enzymes only when population density is high. If the enzymes are produced at low pathogen densities, they might trigger the defense mechanisms of the host plant without being present in sufficient quantity to overwhelm the plant's defenses. Quorum sensing in both *E. carotovora* and *V. fischeri* is mediated by *N*-acyl homoserine lactones, small molecules released from the bacterial cells into the surrounding medium (**Figure OM14.2-2**). At high bacterial densities, the concentration of *N*-acyl homoserine lactone is sufficient to induce the expression of genes encoding luminescent proteins in *V. fischeri* and cell wall-degrading enzymes in *E. carotovora*.



Figure OM14.2-1 Bobtail squid, *Euprymna berryi*. The light-emitting organs of *E. berryi* are inhabited by the bacterium *Vibrio fischeri* and light emission is controlled by a quorum sensing mechanism. (Courtesy of Scubazoo/Science Photo Library.)

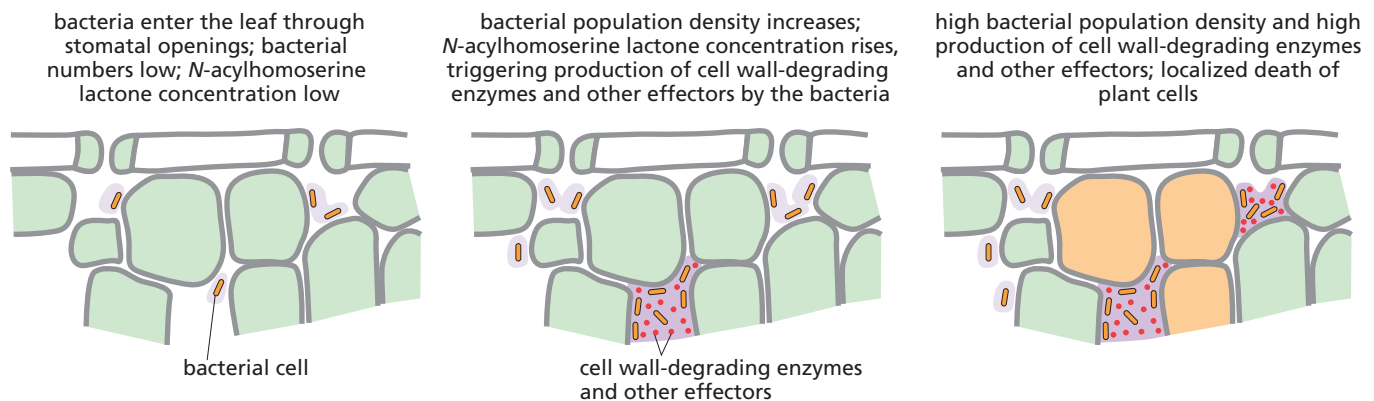


Figure OM14.2-2 Quorum sensing during growth of a pathogenic bacterium in leaf intercellular spaces. Bacteria enter the leaf through stomatal apertures. Early in colonization, the bacterial population inside the leaf is sparse and the *N*-acyl homoserine lactone concentration is low. As the density of the bacterial population rises, the elevated *N*-acyl homoserine lactone concentration leads to expression of cell wall-degrading enzymes. Production of high concentrations of enzymes and other effector molecules results in death of the plant cells (in orange).

OM14.3 MYCOTOXINS AND HUMAN HEALTH

Outbreaks of a convulsive disease known as St Anthony's fire in Europe in the Middle Ages were caused by **ergot** (*Claviceps purpurea*), a fungus that infects grasses and cereal crops including rye (*Secale cereale*) (Figure OM14.3-1A, B). Toxins from the fungus – including the alkaloid ergotamine and related compounds similar in structure to lysergic acid diethylamide (LSD) – contaminated rye flour and hence the bread made from it. *Aspergillus flavus*, a fungus that infects peanuts, produces aflatoxin, a potent cause of liver damage and a carcinogen in humans. The fungi *Fusarium* and *Aspergillus* species that infect the ears of wheat, the cobs of maize, and damp stored grain, produce several dangerous toxins. For example, *Fusarium* species make deoxynivalenol (vomitoxin; Figure OM14.3-1C, D), zearalenones (steroid hormone mimics that can cause testicular feminization in pigs), and fumonisins (associated with high rates of esophageal cancer in humans). Grain crops susceptible to *Fusarium* infection are routinely tested for mycotoxin contamination.

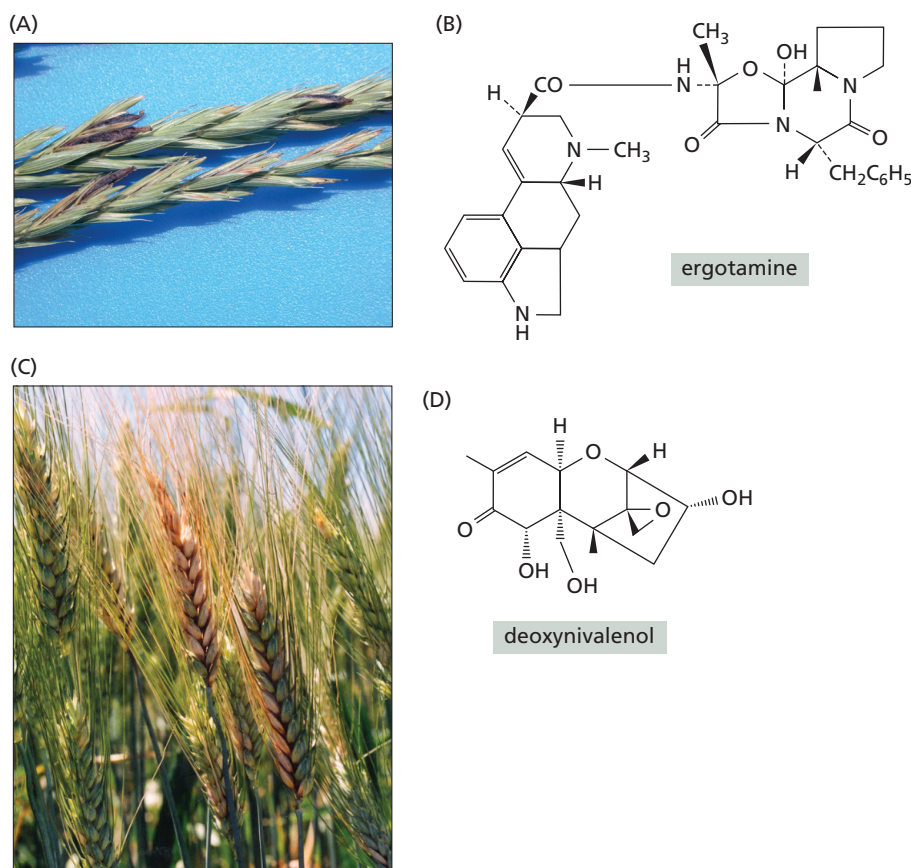


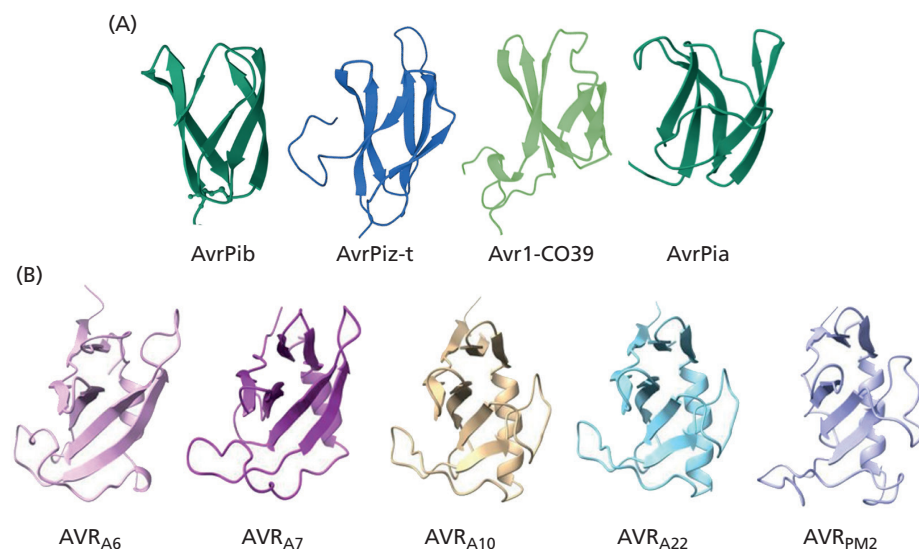
Figure OM14.3-1 Infection by fungi *Claviceps* and *Fusarium*. (A) An ear of the grass *Elymus repens* infected by *Claviceps purpurea*; the large black grains contain spores of the fungus. (B) Structure of ergotamine, a toxin produced by *C. purpurea*, which is harmful to humans. (C) *Fusarium* head blight of wheat. (D) Structure of deoxynivalenol, a dangerous mycotoxin made by *Fusarium graminearum*, the causal agent of *Fusarium* head blight of wheat. (A, courtesy of David Barker, Ohio State University. C, from "CSIRO SciencelImage 11243 *Fusarium* head blight of barley" by CSIRO. Licensed under CC BY 3.0 via Wikimedia Commons.)

OM14.4 DISCOVERY OF POTENTIAL EFFECTOR PROTEINS

Recognized effectors were first cloned using genetic methods, exploiting the gene-for-gene relationship between plant *R* (*resistance*) genes and bacterial *Avr* (*avirulence*) genes that encode recognized effector proteins (described in Section 15.4). These methods led to the discovery that the effector proteins of oomycetes share specific amino acid motifs. For example, oomycete effectors that carry a signal peptide and two additional motifs, the RxLR (arginine, any amino acid, leucine, arginine) and DEER (glutamate, aspartate, aspartate, arginine) motifs, were found to be common to downy mildew (*Peronospora*) and potato late blight (*Phytophthora*) effector proteins. Similarly, recognized effector proteins with specific features in common were identified from the fungal pathogens *Magnaporthe oryzae* and *Blumeria graminis*.

The availability of whole genome sequences for oomycete and fungal pathogens has enabled searches for genes encoding proteins with motifs like those of the recognized effectors. Many potential new effectors have been uncovered in this way. This approach has also shown that many oomycete effectors not only share small, specific motifs but frequently also possess multiple repeats of domains containing WY (tryptophan, tyrosine) or LWY (leucine, tryptophan, tyrosine) motifs that specify the host proteins with which they interact.

Recent advances in the prediction of protein structures from amino acid sequences (such as the artificial intelligence (AI)-based **AlphaFold**) are providing much new information about the common features of effectors. For example, using the available structures for oomycete effectors, AlphaFold can predict new effector protein structures from amino acid sequences that have not yet been investigated experimentally. This technique is providing extensive new insights into the range of virulence factor structures secreted into plant hosts by pathogens. It shows that although pathogen effectors show extreme sequence diversity, they fall into a limited number of structural classes. Effectors with highly divergent sequences may be very similar in general structure. Similarly, effector proteins from the fungal pathogens *M. oryzae* and *B. graminis* (powdery mildew) often share, respectively, either a “MAX effector” protein fold or a fold suggesting evolution from an RNAase (**Figure OM14.4-1**). For more information, see Further Reading.



Further reading

Seong K, Krasileva KV (2023) Prediction of effector protein structures from fungal phytopathogens enables evolutionary analyses. *Nat Microbiol* 8:174–187.

Figure OM14.4-1 Similar protein folds in effectors with diverse sequences. (A) Structures of four effectors (recognized by various rice NLR immune receptors) from the rice blast fungal pathogen *Magnaporthe oryzae*. Although the proteins have little identity at the level of amino acid sequence, they share a “ β -strand sandwich” structure (ribbon arrows represent β -strands). This structure is shared by many other putative effector proteins in members of the genus *Magnaporthe*, and effectors from other ascomycete fungal pathogens such as AvrToxB from the wheat tan spot pathogen *Pyrenophora tritici-repentis*. These structurally conserved proteins are collectively grouped as MAX (*Magnaporthe* Avr ToxB) effectors. They presumably had a common evolutionary origin that was subject to diversifying selection to evade host recognition while retaining function. (B) Structures of effectors from the ascomycete powdery mildew pathogen *Blumeria graminis*. Resistance to powdery mildew in barley is brought about by NLR immune receptors encoded at the *Mla* resistance locus. Each NLR allele (variant) recognizes a different *B. graminis* effector. Although these examples of *B. graminis* effectors (AVR_{A6}, AVR_{A7}, AVR_{A10}, and AVR_{A22} in the figure), and an effector produced during *B. graminis* infection of wheat (AVR_{PM2} in the figure) have little sequence identity, they all have very similar structures (ribbon arrows represent β -strands, corkscrew ribbons represent α -helices). (B, from Kümmel F *et al.* (2023) *Proc Natl Acad Sci USA* 120:e2307604120. Copyright © 2023 the Author(s). Published by PNAS. Distributed under CC BY 4.0.)

OM14.5 SPECIFICITY OF DNA BINDING BY TAL EFFECTORS

The DNA sequence bound by a particular transcription activator-like (TAL) protein is specified by unique amino acid sequences in variable repeats of 34 amino acids in the protein. The greatest point of variation (polymorphism) between the repeats occurs in the amino acids at positions 12 and 13 in each repeat. This pair of amino acids – referred to as the repeat variable di-residue (RVD) – recognizes a specific nucleotide in the DNA to which the TAL binds. Thus, as shown in **Figure OM14.5-1**, a TAL with 15 repeats (thus containing 15 RVDs) recognizes a precise DNA sequence of 15 nucleotides. Computational analysis and functional studies have “cracked the code” that links the RVDs to DNA sequence. The nucleotide adenine is recognized by the amino acid pair (RVD) asparagine-isoleucine, guanine is recognized by asparagine-asparagine, cytosine is recognized by histidine-aspartic acid, and thymine is recognized by asparagine-glycine.

The discovery of this code has enabled elucidation of the sequences in the host genome of each type of TAL: the amino acid sequence of the variable region of the TAL can be “translated” into a DNA sequence which can then be located by scanning the host genome. This knowledge also has biotechnological potential. It can be used to design new variable repeats to target any DNA sequence of interest, and to design new promoter regions for reporter or defense genes that will be activated when TALs enter the plant. Note the parallels between the specificity of TAL binding to DNA sequences and the specificity of binding of pentatricopeptide repeat (PPR) proteins to RNA sequences during plastidial RNA editing (see Section 3.4).

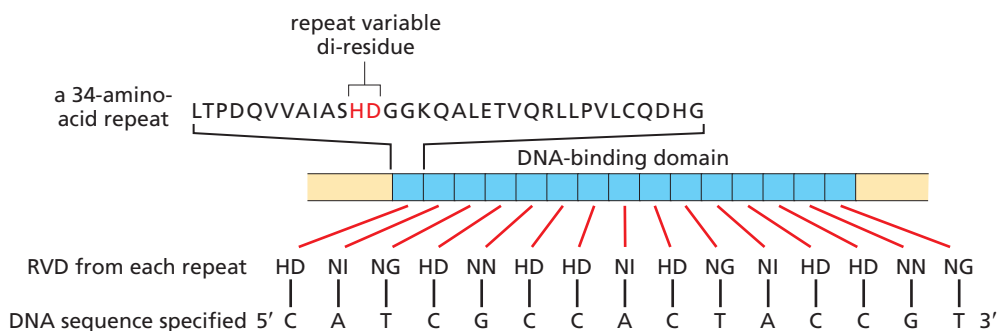


Figure OM14.5-1 Precise binding of a specific DNA sequence by a 15-repeat TAL protein.

OM14.6 PLANT TRANSFORMATION USING T-DNA

In the most commonly used system for plant transformation in research and biotechnology labs, the *vir* genes and the T-DNA components of the Ti plasmid are separated onto two different plasmids that can be propagated in the same bacterial cell, creating a **binary vector system** (Figure OM14.6-1).

The T-DNA is carried on a plasmid that can replicate in *Agrobacterium* and in *Escherichia coli* (the bacterium used in laboratories for DNA manipulation and replication). The T-DNA retains its left and right borders, but the genes encoding enzymes of opine and hormone biosynthesis are deleted (a “disarmed” vector). Instead, the T-DNA carries a “**selectable marker**” gene, which usually encodes a protein that confers resistance to an antibiotic or other compound that is normally toxic to plant cells. This marker enables researchers to select specifically the plant cells that have received T-DNA molecules; these transformed cells will be able to grow on substrates containing the toxic compound but untransformed cells will be killed. Additional genes or other DNA sequences of interest to researchers can be cloned into the T-DNA region between the left and right borders next to the selectable marker.

The binary vector system is assembled in a strain of *Agrobacterium* that carries a Ti plasmid lacking the T-DNA region but still carrying the *vir* genes that act *in trans* to enable T-DNA delivery. The *Agrobacterium* is then able to infect plant cells. Subsequent cultivation of the cells on a substrate containing the appropriate toxic compound selects for transformed material that can be regenerated to give whole, transgenic plants (Figure OM14.6-2). Alternatively, direct transformation of the female gametophyte (via “**floral dipping**,” the immersion of inflorescences in an *Agrobacterium* suspension for *A. thaliana* transformation) with the chimeric T-DNA confers heritable antibiotic resistance on the progeny. Whatever the method of transformation, transformed plants, identified because they express antibiotic resistance, are subsequently tested for expression of the characteristic conferred by the novel gene of interest introduced into the plant genome. This technique has been widely exploited both in plant research and in biotechnology: examples are given throughout this book.

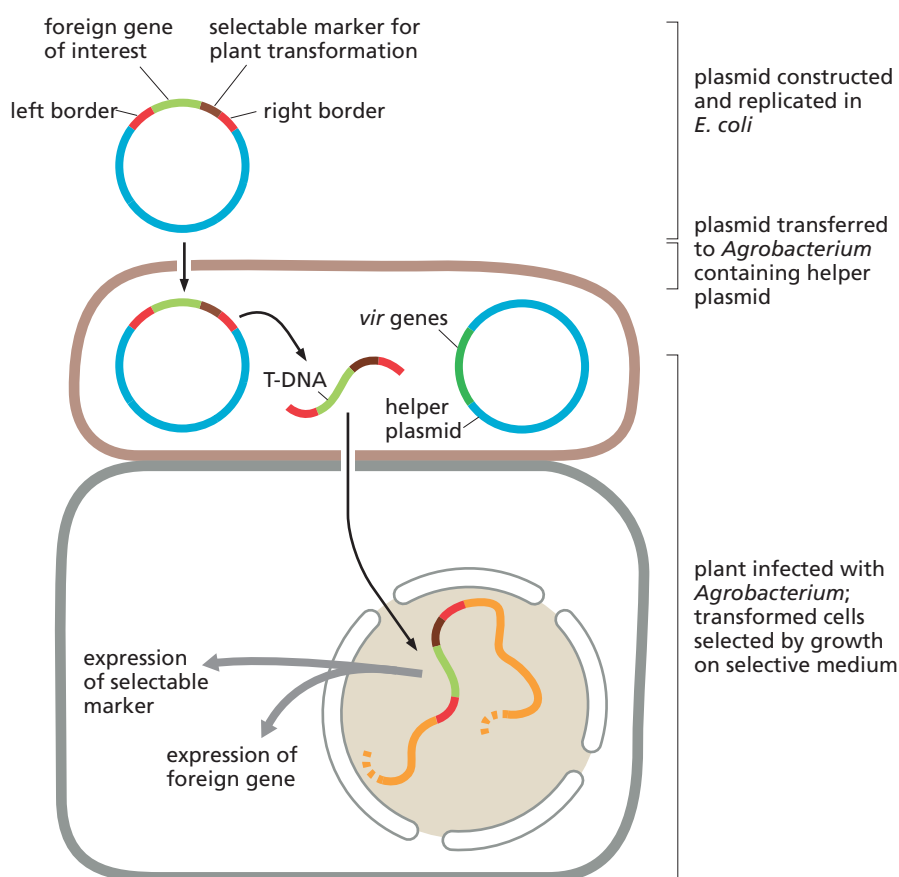


Figure OM14.6-1 Use of a binary vector system for plant transformation.

Using standard techniques for DNA manipulation in the bacterium *Escherichia coli*, researchers engineer a T-DNA that contains the foreign gene of interest, a selectable marker gene, and the left and right borders necessary for precise T-DNA excision. The selectable marker gene encodes a protein that enables detoxification of a compound normally poisonous to plant cells. (For example, a commonly used selectable marker gene encodes an enzyme that modifies and detoxifies the antibiotic **kanamycin**.) A plasmid containing this engineered T-DNA is transferred to a strain of *Agrobacterium* containing a second, “helper” plasmid with the *vir* genes necessary for T-DNA transfer to the plant cell and integration into a plant chromosome. The *Agrobacterium* strain containing the two plasmids is used to infect plant tissue. The infected tissue is grown on a selective medium – that is, a medium containing the toxic compound (e.g. kanamycin) that can be detoxified by the protein encoded by the selectable marker gene in the T-DNA. Plant cells that have not been infected are killed by the toxic compound in the medium. Cells that receive DNA from *Agrobacterium* – and thus containing the foreign gene of interest – can detoxify the toxic compound and are able to grow. Transformed plants expressing the foreign gene can be regenerated from these cells.

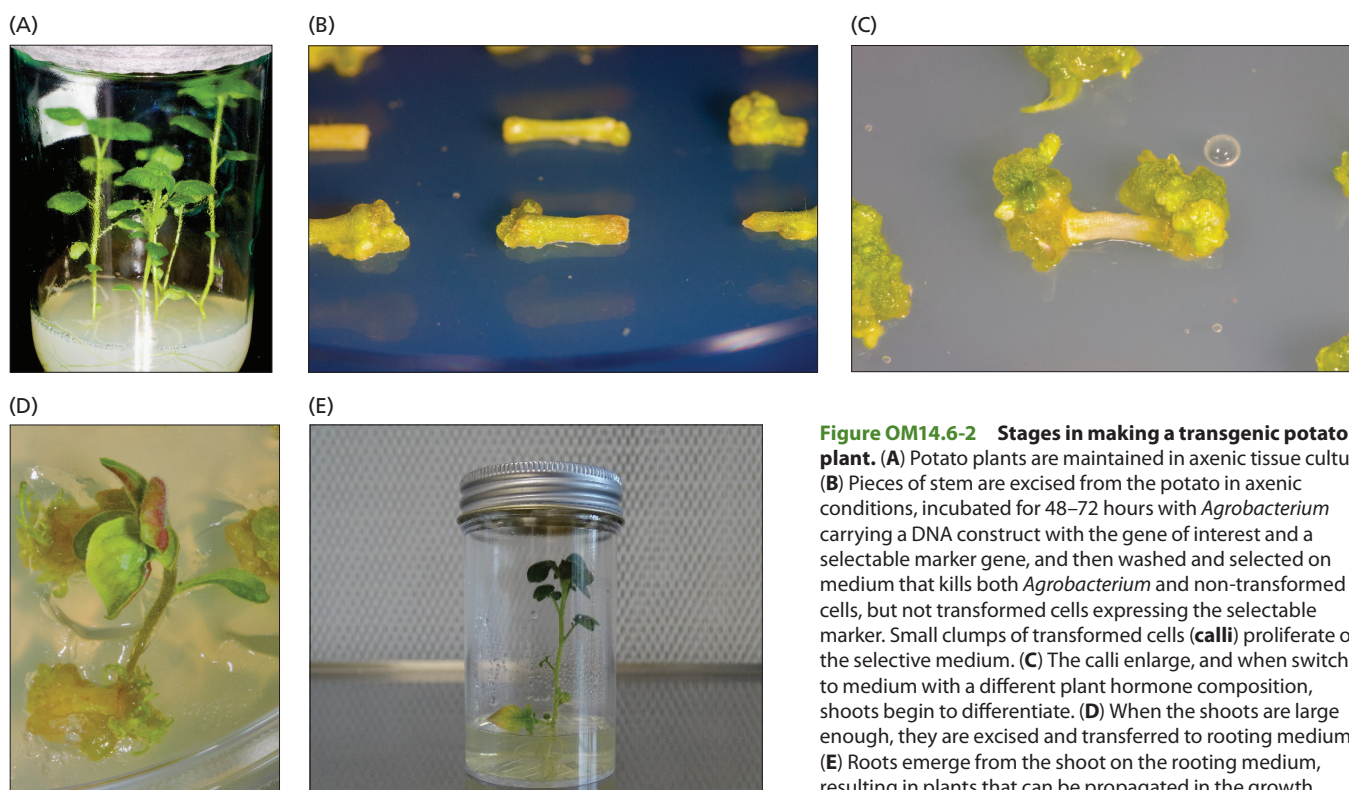


Figure OM14.6-2 Stages in making a transgenic potato plant. (A) Potato plants are maintained in axenic tissue culture. (B) Pieces of stem are excised from the potato in axenic conditions, incubated for 48–72 hours with *Agrobacterium* carrying a DNA construct with the gene of interest and a selectable marker gene, and then washed and selected on medium that kills both *Agrobacterium* and non-transformed cells, but not transformed cells expressing the selectable marker. Small clumps of transformed cells (**calli**) proliferate on the selective medium. (C) The calli enlarge, and when switched to medium with a different plant hormone composition, shoots begin to differentiate. (D) When the shoots are large enough, they are excised and transferred to rooting medium. (E) Roots emerge from the shoot on the rooting medium, resulting in plants that can be propagated in the growth chamber or greenhouse.

A. tumefaciens usually infects eudicots, and not monocots. When *Agrobacterium*-based plant transformation systems were first developed, they were suitable only for eudicots. However, by experimenting with different *Agrobacterium* strains, and by manipulating *vir* gene expression, extremely efficient transformation systems have been devised for many monocots, including the important cereal crops maize, rice, wheat, and barley.

The choice of promoter for the chimeric gene construct described depends on the level or the tissue specificity of gene expression that is required and on the species that is being transformed. The cauliflower mosaic virus (CaMV) 35S promoter is widely used. It drives high-level gene expression in the tissues of most eudicots, but works only poorly in most monocots. For this reason, a maize ubiquitin gene promoter is commonly used for expression of genes in monocots. Other promoters that are expressed at precisely defined developmental stages, in response to external stimuli, or in specific cell types, can be used to direct expression of the chimeric gene accordingly.

Agrobacterium transformation can also be used to achieve transient expression of genes of interest. This technique (often referred to as **agroinfiltration**) is usually used on the tobacco species *Nicotiana benthamiana* and *N. tabacum*, to discover the function or interactions of the protein product of a gene or genes of interest, or to generate large amounts of the encoded protein for further studies (Figure OM14.6-3). It can also be used to assemble novel metabolic pathways that synthesize valuable metabolites. In overview, a suspension of *Agrobacterium* in which the T-DNA encodes the gene(s) of interest is infiltrated into leaves of an intact plant. This can be achieved on a small scale by injecting the suspension into the leaf surface using a syringe with no needle, or on a larger scale by briefly immersing the whole plant in the suspension under a vacuum. After a few days, the infiltrated regions express the protein(s) encoded on the T-DNA. Transient expression can provide rapid tests for gene function without the need to regenerate transgenic plants. Examples are shown in Figure OM14.6-3.

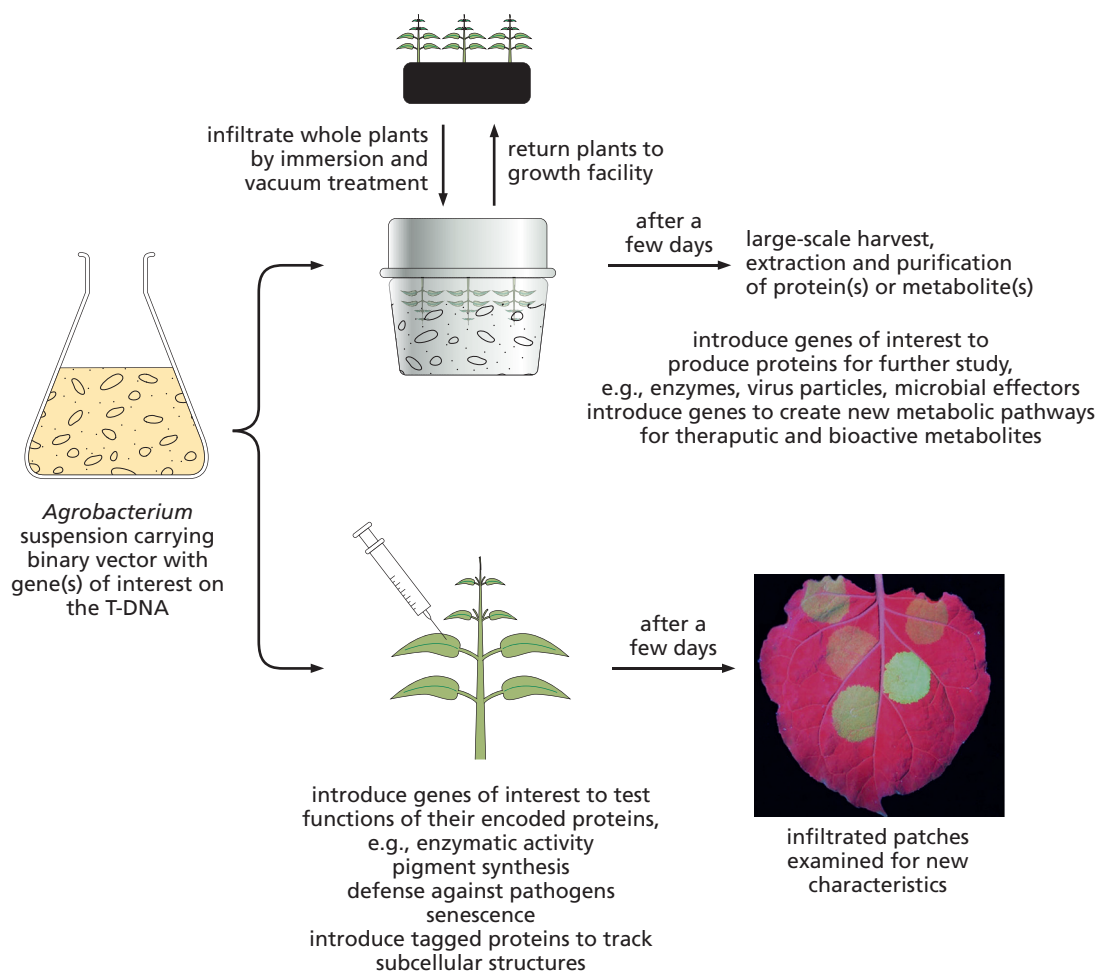


Figure OM14.6-3 Agroinfiltration techniques. (Top) Large-scale agroinfiltration, where plants are inverted into a suspension of *Agrobacterium* carrying a binary vector with gene(s) of interest on the T-DNA. Infiltration of the bacteria into the leaf is achieved by brief application of a vacuum to the immersed shoots. Plants are removed, and after a few days of growth the leaves express large amounts of the protein(s) encoded by the introduced genes. The proteins or the products of the metabolic pathway they catalyze can be extracted from harvested leaves and purified. (Bottom) Small-scale agroinfiltration, where the *Agrobacterium* suspension is introduced into regions of individual leaves. This is often achieved by applying gentle pressure to the leaf with a loaded syringe with no needle. After a few days, the infiltrated region of the leaf expresses the introduced gene. Small-scale agroinfiltration experiments often employ fusion proteins – the protein of interest “tagged” with a protein that can easily be visualized such as green fluorescent protein (GFP) or luciferase. This enables the rapid assessment of amount or effects of the protein of interest. An example of GFP fluorescence from a transiently expressed fusion protein is shown (right). The red background is due to chlorophyll fluorescence. (Photo, from Ma X *et al.* (2019) *Virology* 16:20. Distributed under CC BY 4.0.)

OM14.7 INVASION OF HOST-PLANT ROOTS BY HAUSTORIA OF *STRIGA*

The haustoria of plant parasites are multifunctional organs that attach the parasite to the host, invade host tissues, and provide vascular continuity between the host and parasite through which the parasite gains water and nutrients. Analysis of haustorium development is helped greatly by the fact that it can be studied in the laboratory in the absence of a host plant. The roots of *Striga* seedlings respond rapidly to application of exudates from host-plant roots. Localized swelling of *Striga* root tips, leading to the formation of haustoria, occurs within hours, caused initially by expansion and later by division of cortical cells. At the same time, there is a proliferation of root hairs over the swollen zone of the *Striga* root (Figure OM14.7-1A). These hairs differ from typical root hairs in that their surface is coated with hemicellulose-rich protrusions (papillae) that promote attachment of the hairs, and hence the haustoria, to host-cell surfaces.

Although *Striga* haustoria can attach to the roots of non-host species, successful penetration of the root occurs only on host species. Attachment of haustoria to the roots of non-host plants elicits defense responses similar to those elicited by microbial pathogens (described in Chapter 15). On host plants, haustorial cells at the parasite–host interface elongate and divide, pushing through the epidermis and cortex of the host root. When *Striga* seedlings are placed on sorghum roots, they can penetrate through the cortex to reach the vascular tissue in the stele within 2–3 days (Figure OM14.7-1B).

Once a haustorium has penetrated through to the stele, the leading cells enter host xylem vessels through the pits in the xylem walls (see Section 8.3). Haustorial cells lose their cell walls at the tips, and these cells and adjacent cortical cells in the parasite root differentiate to form a continuous water-conducting system that links the host xylem with the vasculature of the parasite root (Figure OM14.7-1C).

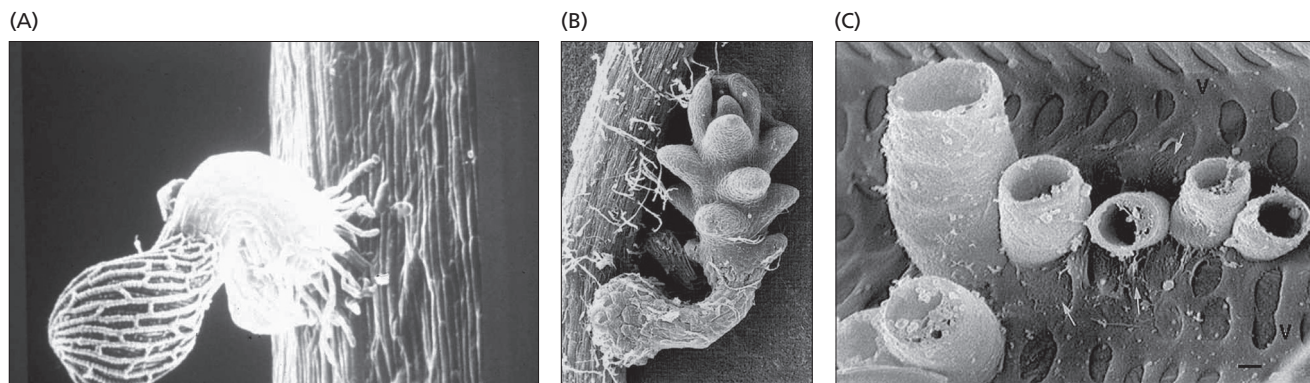


Figure OM14.7-1 Scanning electron micrographs showing *Striga* infection and development. (A) A *Striga* seed (the structure with a netted surface) after germination adjacent to the root of a host plant. The seedling root has formed a haustorium on the host root surface; the haustorium is surrounded by root hairs that promote its attachment to the root surface. (B) A later stage in *Striga* development. The shoot is starting to develop, and attachment to the xylem of the host root is established. (C) The inner face of a xylem vessel wall in a host-plant root, showing the tube-like water-conducting vessels (oscula) of a *Striga* plant penetrating the wall. (B, C, from Dorr I (1997) *Ann Bot* 79:463–472. Reprinted by permission of Oxford University Press.)

OM14.8 BIOLOGY OF PLANT VIRUSES

Viral classification is based on a range of factors that include viral particle size and shape, the nature of the viral genome, and the different strategies for viral replication and protein production. Plant viruses are most often spherical (isometric) or rod-shaped with a straight or wavy morphology. The nucleic acid of plant viruses may be RNA or DNA, and it may be single-stranded (ss) or double-stranded (ds), depending on the type of virus. The majority (about 70%) of plant viruses have genomes of ssRNA with coding potential (+ sense); viral particles containing complementary (-) strand RNA also occur. This section describes the general features of three major groups of viruses: (i) single-stranded (+) RNA viruses, exemplified by tobamoviruses and potyviruses; (ii) dsDNA viruses, exemplified by caulimoviruses; and (iii) ssDNA viruses, exemplified by geminiviruses. The discussion considers the salient features of their structures and replication mechanisms, and how the viruses might cause disease symptoms. Plant defenses against viruses are described Chapter 15.

Different types of plant viruses have different structures and replication mechanisms

The type member of the tobamovirus group is tobacco mosaic virus (TMV). (A type member of a group or taxon in biology is the representative member or specimen from the group that epitomizes the key features of the group.) TMV is a simple, single-stranded (+) RNA (meaning it can function as an mRNA for the production of virus-encoded proteins), straight rod-shaped virus (**Figure OM14.8-1A**) that is transmitted mechanically (for example through wounds on the plant surface) rather than via a vector. The multiplication of the virus occurs in several steps (**Figure OM14.8-1B**). After entry into the plant cell, the virus must be uncoated before the RNA can be translated. For TMV this process is coupled with the first step of translation such that the ribosome binds to the 5' end of the viral RNA and displaces coat protein subunits as the RNA is read. The first protein to be produced is the RNA-dependent RNA polymerase, which uses the viral RNA molecule as a template on which to synthesize complementary (-) RNA, a process that initiates at the 3' end of the (+) strand RNA. The (-) strands produced by the RNA polymerase serve two purposes. First, parts of the (-) strand are copied again by RNA polymerase, but starting at an internal site and so producing smaller subgenomic (+) RNA molecules, each of which encodes a subset of the viral proteins. These subgenomic RNAs are in turn translated to give specific viral proteins. Second, the whole (-) strand is used as a template by a viral RNA polymerase to produce new, complete (+) strands. These associate with the coat proteins produced by translation of the subgenomic viral RNA to form new viral particles.

The TMV genome encodes only four proteins. The two largest (126 and 183 kDa) are components of the RNA polymerase. The other two comprise a coat protein (18 kDa) and a movement protein (30 kDa). The movement protein changes the size exclusion limit of plasmodesmata (see Section 8.2; the limit is normally about 1 kDa) to allow passage of larger particles, facilitating the movement of a TMV RNA-movement protein complex from cell to cell through the host plant. Because the virus encodes so few proteins, it is dependent on components of the host cell for many aspects of its replication.

The type member of the potyvirus group is potato virus Y (PVY). The particles of this virus are flexuous rods and, like TMV, have a single-stranded (+) RNA genome (see Figure 14.25B). Potyviruses are more complex than tobamoviruses. The extra complexity is of two sorts. Potyviruses encode more proteins than TMVs, and their RNAs are translated to produce large polyproteins that are cleaved by viral proteases to form the active viral proteins. For example, the approximately 10 kb genome of PVY RNA is translated into a single polyprotein of about 360 kDa that is subsequently cleaved at specific sequences to produce, ultimately, about ten proteins, each of which has separate and often multiple functions. In addition to the components of the viral RNA polymerase, coat proteins, and movement proteins, potyviruses encode proteases, an aphid transmission factor, and the protein VPg, which is attached to the 5' end of the RNA molecule and is required for initiation of replication and for translation. Although the basic principles of RNA replication for potyviruses are similar to those for tobamoviruses, potyviruses do not produce subgenomic RNAs – hence the different translation mechanism. Another group of viruses, the comoviruses that include cowpea mosaic virus (see Figure 14.25A), have a translation mechanism somewhat similar to that of potyviruses.

The type member of the caulimovirus family is cauliflower mosaic virus (CaMV). This virus is spherical and has a genome composed of circular dsDNA. The caulimovirus family falls within the larger badnavirus family, which collectively is unusual in that

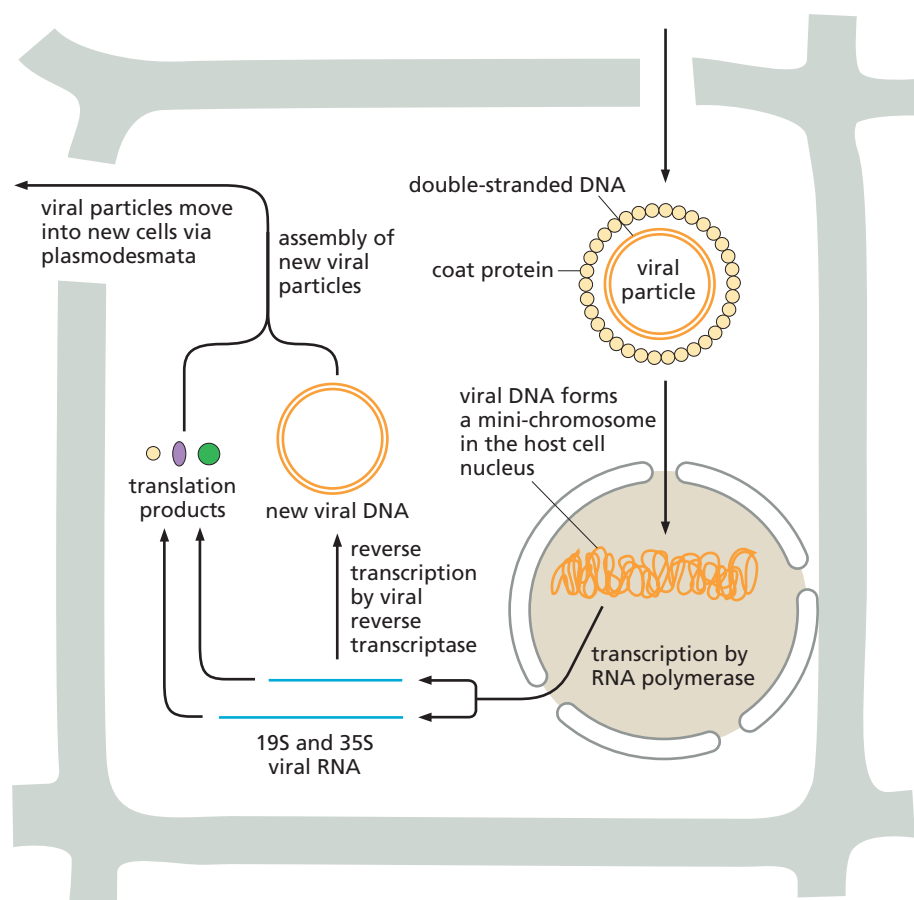


Figure OM14.8-2 Replication of cauliflower mosaic virus. When the viral particle enters a plant cell (*top right*), coat proteins are removed and the double-stranded DNA enters the nucleus, where it forms a mini-chromosome. This is transcribed to produce a 19S and a 35S RNA. The RNAs are translated to produce new proteins (as described in the text), including a movement protein for cell-to-cell transmission, a reverse transcriptase, and coat protein required for the assembly of new viral particles. The reverse transcriptase synthesizes the complementary DNA strand from the viral RNA, and then converts this to dsDNA, which is assembled into new viral particles.

The geminiviruses are a large family of viruses that carry one or more small, circular ssDNA genomes of between 2.7 and 5.4 kb (see Figure 14.25C). The name “geminiviruses” (Latin for “twins”) refers to the appearance of the viral particles, which comprise two fused, partially isometric subparticles (Figure OM14.8-3A). Geminiviruses encode factors required to initiate replication of their ssDNA genomes, but – unlike the other viruses discussed here – they do not encode replicases (nucleic acid polymerases). They rely instead on host-plant nuclear DNA polymerases to replicate their genomes. However, differentiated, non-dividing plant cells normally lack DNA polymerase and the associated factors required for replication. The presence of geminiviruses induces the expression of the host genes encoding these factors, allowing the virus to replicate. This induction is achieved by inhibition of retinoblastoma (Rb) protein, a negative regulator of the cell cycle (see Section 8.2). Inhibition of Rb allows expression of transcription factors that induce differentiated cells to re-enter the cell cycle. The viral genome is then replicated by the induced DNA replication machinery of the host plant.

When geminivirus DNA enters a cell, the ssDNA is converted to dsDNA in the nucleus. The dsDNA is used as a template for transcription, and for DNA replication by a rolling-circle replication mechanism to produce more ssDNA genomes for encapsidation (Figure OM14.8-3B). Proteins are encoded on both strands of the DNA, and transcription must be bidirectional to produce RNAs for all the encoded proteins.

Geminiviruses are found mostly in warmer climates, probably because of the geographic restrictions in the distribution of their leafhopper and whitefly insect vectors. Examples include wheat dwarf virus and maize streak virus, both transmitted by leafhoppers; tomato yellow leaf curl virus, a whitefly-transmitted virus that causes a serious disease of tomato crops; and the whitefly-transmitted African cassava mosaic virus, which causes devastating losses in cassava crops.

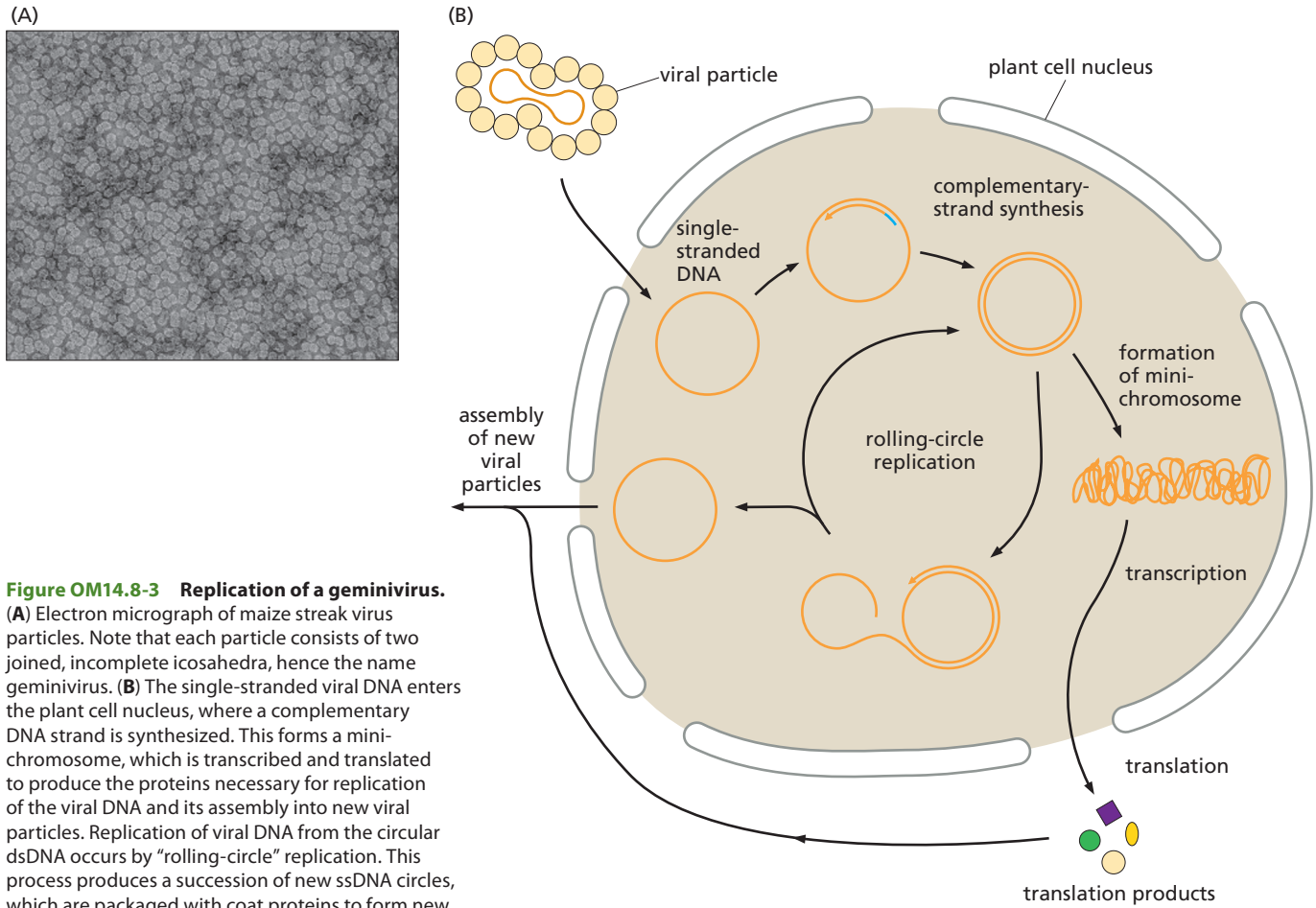


Figure OM14.8-3 Replication of a geminivirus. (A) Electron micrograph of maize streak virus particles. Note that each particle consists of two joined, incomplete icosahedra, hence the name geminivirus. (B) The single-stranded viral DNA enters the plant cell nucleus, where a complementary DNA strand is synthesized. This forms a mini-chromosome, which is transcribed and translated to produce the proteins necessary for replication of the viral DNA and its assembly into new viral particles. Replication of viral DNA from the circular dsDNA occurs by “rolling-circle” replication. This process produces a succession of new ssDNA circles, which are packaged with coat proteins to form new viral particles. Although virally encoded proteins are required for rolling-circle DNA replication, the DNA polymerase is of plant origin. (A, courtesy of Margaret Boulton.)