# VIROLOGY

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Unit 3, Viral transmission, salient features of viral nucleic acids and replication

Understanding how infectious pathogens spread is critical to preventing infectious disease. Many pathogens require a living host to survive, while others may be able to persist in a dormant state outside of a living host. But having infected one host, all pathogens must also have a mechanism of transfer from one host to another or they will die when their host dies. Pathogens often have elaborate adaptations to exploit host biology, behaviour, and ecology to live in and move between hosts. Hosts have evolved defences against pathogens, but because their rates of evolution are typically slower than their pathogens (because their generation times are longer), hosts are usually at an evolutionary disadvantage. This section will explore where pathogens survive—both inside and outside hosts—and some of the many ways they move from one host to another.

**Reservoirs and Carriers**

For pathogens to persist over long periods of time they require **reservoirs** where they normally reside. Reservoirs can be living organisms or non-living sites. Non-living reservoirs can include soil and water in the environment. These may naturally harbour the organism because it may grow in that environment. These environments may also become contaminated with pathogens in human feces, pathogens shed by intermediate hosts, or pathogens contained in the remains of intermediate hosts.

Pathogens may have mechanisms of dormancy or resilience that allow them to survive (but typically not to reproduce) for varying periods of time in non-living environments. Although many viruses are soon destroyed once in contact with air, water, or other non-physiological conditions, certain types are capable of persisting outside of a living cell for varying amounts of time. For example, a study that looked at the ability of **influenza viruses** to infect a cell culture after varying amounts of time on a banknote showed survival times from 48 hours to 17 days, depending on how they were deposited on the banknote. On the other hand, cold-causing **rhinoviruses** are somewhat fragile, typically surviving less than a day outside of physiological fluids.

A human acting as a reservoir of a pathogen may or may not be capable of transmitting the pathogen, depending on the stage of infection and the pathogen. To help prevent the spread of disease among school children, the Centre for disease control and prevention has developed guidelines based on the risk of transmission during the course of the disease. For example, children with chickenpox are considered contagious for five days from the start of the rash, whereas children with most gastrointestinal illnesses should be kept home for 24 hours after the symptoms disappear.

An individual capable of transmitting a pathogen without displaying symptoms is referred to as a carrier. A **passive carrier** is contaminated with the pathogen and can mechanically transmit it to another host; however, a passive carrier is not infected. For example, a health-care professional who fails to wash his hands after seeing a patient harboring an infectious agent could become a passive carrier, transmitting the pathogen to another patient who becomes infected.

By contrast, an **active carrier** is an infected individual who can transmit the disease to others. An active carrier may or may not exhibit signs or symptoms of infection. For example, active carriers may transmit the disease during the **incubation period** (before they show signs and symptoms) or the **period of convalescence** (after symptoms have subsided). Active carriers who do not present signs or symptoms of disease despite infection are called **asymptomatic carriers**. Pathogens such as **hepatitis B virus**, **herpes simplex virus**, and **HIV** are frequently transmitted by asymptomatic carriers.

**Transmission**

Regardless of the reservoir, **transmission** must occur for an infection to spread. First, transmission from the reservoir to the individual must occur. Then, the individual must transmit the infectious agent to other susceptible individuals, either directly or indirectly. Pathogenic microorganisms employ diverse transmission mechanisms.

**Contact Transmission**

**Contact transmission** includes direct contact or indirect contact. **Person-to-person transmission** is a form of **direct contact transmission**. Here the agent is transmitted by physical contact between two individuals through actions such as touching, kissing, sexual intercourse, or **droplet sprays**. Direct contact can be categorized as vertical, horizontal, or droplet transmission.

When an individual coughs or sneezes, small droplets of mucus that may contain pathogens are ejected. This leads to direct **droplet transmission**, which refers to droplet transmission of a pathogen to a new host over distances of one meter or less. A wide variety of diseases are transmitted by droplets, including **influenza** and many forms of **pneumonia**. Transmission over distances greater than one meter is called **airborne transmission**.

**Indirect contact transmission**

**Indirect contact transmission** involves inanimate objects called **fomites** that become contaminated by pathogens from an infected individual or reservoir. For example, an individual with the common cold may sneeze, causing droplets to land on a fomite such as a tablecloth or carpet, or the individual may wipe her nose and then transfer mucus to a fomite such as a doorknob or towel. Transmission occurs indirectly when a new susceptible host later touches the fomite and transfers the contaminated material to a susceptible portal of entry. Fomites can also include objects used in clinical settings that are not properly sterilized, such as syringes, needles, catheters, and surgical equipment. Pathogens transmitted indirectly via such fomites are a major cause of healthcare-associated infections

**Horizontal Transmission**

**At its simplest, transmission is defined as the means by which an infectious agent is passed from an infected host to a susceptible host**. It is a function of both the host and the pathogen and consists of pathogen presentation by the host, movement between infected and healthy hosts, and entry into the new host. Transmission dynamics may involve varying degrees of complexity, from single-host species (measles- or rubella viruses) to contrasting multiple host species (Rift Valley fever phlebovirus, Phenuiviridae). Further, viruses are able to use, simultaneously or sequentially, multiple modes of transmission, including but not exclusive to vertical or horizontal transmission. In vertical transmission, viruses are passed vertically from mother to offspring. In horizontal transmission, viruses are transmitted among individuals of the same generation which encompasses both direct and indirect modes. Horizontal transmission can be further classified as direct or indirect. Horizontal transmission by a direct route includes airborne infection, foodborne infection, and venereal (sexual) infection, whereas transmission by an indirect route involves an intermediate or an inanimate object (fomite) or a biological host, like a mosquito vector, which acquires and transmits virus from one host to another. Table 1 summarizes the known modes of transmission of viruses. Viruses that use biological vectors are given in Table 2.

**Airborne Viruses**

Like cellular microorganisms, viruses are ubiquitously found in the air. Viruses can become airborne only if conditions for aerosolization are met (mass, size, shape, and density, among others). An aerosol is a particle suspension in a gaseous medium, e.g., the air. A virus can be naturally aerosolized primarily by sneezing, or secondarily, when an infected surface serves as the source of air transportation by means of other mechanical processes, like splashing, bubbling, sprinkling or even toilet flushing. Human (seasonal) behaviour (personal hygiene, closed environments, densely populated areas, transportation hubs, and pollution) is regarded as an important contributor to the spread of viruses by aerosols. Additionally, viruses can be aerosolized by coughing flying animals (bats and birds, for instance); droppings can also serve as a primary source of viruses than can later be secondarily transmitted by air. In the case of Foot-and-mouth disease virus (Picornaviridae), e.g., computer simulations have estimated that “in a worst-case scenario” cattle could be infected as far as 20300 km far from the infectious source.

Table 1, mode of viral transmission

| Viral Family | Hosts[a] | Transmission | Vecto |
|---|---|---|---|
| *Adenoviridae* | A | Respiratory droplets; orofecal route | N |
| *Alloherpesviridae* | A | Passive diffusion | N |
| *Alphaflexiviridae* | F–P | Mechanical; vector; grafting; others unknown | Y/N |
| *Alphatetraviridae* | A | Oral route. Vertical transmission also possible | N |
| *Amalgaviridae* | P | Vertically through seeds; horizontally undocumented | ? |
| *Ampullaviridae* | Ar | Passive diffusion | N |
| *Anelloviridae* | A | Sexual, blood, saliva; possibly also orofecal route and maternal transmission | N |
| *Arenaviridae* | A | Zoonosis (saliva, urine, nasal secretions of rodents); fomites; aerosol. Vertically: transuterine, transovarian. Milk, saliva, urine | N |
| *Arteriviridae* | A | Genital and respiratory tract secretions; transplacental; urine, semen; probably contact; aerosol | N |
| *Ascoviridae* | A | Mechanical; vector. Vertically during oviposition | Y |
| *Asfarviridae* | A | Mechanically by biting flies. Contact, fomites, ingestion | Y |
| *Astroviridae* | A | Orofecal route | N |
| *Avsunviroidae* | P | Mechanical. Seeds; vegetative propagation | N |
| *Baculoviridae* | A | Orofecal route; contamination of egg surface. Vertically from infected male or female parent to the egg | N |
| *Barnaviridae* | F | Transmission is horizontal via mycelium and possibly basidiospores | N |
| *Benyviridae* | P | Mechanical; vector | Y |

Table 2, viruses and their vectors

| Virus Family (Genus) | Hosts[a] | Type of Vector (Some Examples) |
|---|---|---|
| *Alphaflexiviridae* (*Allexivirus*) | P | Arachnids: Mites (*Aceria tulipae*) |
| *Alphaflexiviridae* (*Potexvirus*) | P | Insects: Aphids (if a potyvirus provides a helper protein), or buff-tailed bumblebees (*Bombus terrestris*) in greenhouse |
| *Ascoviridae* (*Ascovirus*) | A | Insects: Endoparasitic wasps (e.g., *Campoletis sonorensis, Diadegma semiclausum, Microplitis similis, M. croceipes, Toxoneuron nigriceps*) |
| *Ascoviridae* (*Toursvirus*) | A | Insects: Endoparasitic wasps (e.g., *Diadromus pulchellus*) |
| *Asfarviridae* (*Asfivirus*) | A | Arachnids: Argasid ticks (reservoir) of the genus *Ornithodoros* |
| | | Insects: flies and bugs (genera *Simulium, Stomoxys,* and *Triatoma*) |
| *Benyviridae* (*Benyvirus*) | P | Plasmodiophorids: *Polymyxa betae* and *P. graminis* |
| *Betaflexiviridae* (*Carlavirus*) | P | Insects: Aphids or whiteflies (*Bemisia tabaci*) |
| *Betaflexiviridae* (*Trichovirus*) | P | Arachnids: Mites (*Colomerus vitis, Eriophyes inequalis, E. insidiosus*) |
| *Betaflexiviridae* (*Vitivirus*) | P | Insects: Pseudococcid mealybugs (genera *Pseudococcus* and *Planococcus*), scale insects (*Neopulvinaria innumerabilis*), and aphids |
| *Bromoviridae* (*Alfamovirus*) | P | Insects: Aphids (*Myzus persicae* and at least 13 more species belonging to the family Aphididae) |
| *Bromoviridae* (*Bromovirus*) | P | Insects: Beetles (although with low efficiency). For example, *Diabrotica undecimpunctata howardi* for *Cowpea chlorotic mottle virus* |
| *Bromoviridae* (*Cucumovirus*) | P | Insects: Aphids (genera *Aphis, Myzus*)<br>Fungi: *Cucumber mosaic virus* in *Rhizoctonia solani* |
| *Caulimoviridae* (*Badnavirus*) | P | Insects: Aphids, mealybugs (*Planococcus citri*), and lacebugs |

### Waterborne Viruses

Water is an excellent medium for the transportation and dissemination of viruses. The oceans are a particularly important habitat of bacterial and archaeal viruses—along with those that infect marine plants, animals, fungi, protozoa, and chromista (algae and protozoa having chlorophyll). Water transmitted animal viral pathogens include adeno-, astro-, rota-, noro-, calici-, and polioviruses, as well as hepatitis viruses; urine secreted viruses that can reach water, like polyoma- and cytomegaloviruses, can also be included in the list of water spread viruses. Evidence on the water dissemination of influenza- and coronaviruses is inconclusive

### Soilborne Viruses

Some viruses are extremely stable under soil conditions. In general, virus survival in soils depends mostly on temperature and virion adsorption to soil (sand and clay colloids). Other influential factors include soil moisture content, presence of aerobic microorganisms, levels of resin-extractable phosphorous, exchangeable aluminum, organic matter, and soil pH. TMV (Virgaviridae), for instance, remains infectious in soil for several years in living or dead plant debris. Infections have been reported with plants brought into physical contact with soilborne viruses during transplanting. Although the majority of plant viruses are transmitted by arthropod vectors and invade the host plants through the aerial parts, there is a considerable number of plant viruses that infect roots via soil-inhabiting vectors such as plasmodiophorids, chytrid fungi, and nematodes (see later). Regarding animal viruses, polioviruses (Picornaviridae) are stable in soils provided temperatures are not high (e.g., 3 months at $4^0$C). At low temperatures and a pH of 7.5, some enteroviruses in soil may survive from 110 to 170 days. Influenza A virus (Orthomyxoviridae) H5N1 can be found in soilbased composts.

**Transplantation, Anastomosis, and Grafting**

Of equal significance is the transmission of viruses via allotransplantation. A variety virus may be transmitted by this route and include Human immunodeficiency virus 1, West Nile virus, Human betaherpesvirus 5, Rabies lyssavirus as well as hepatitis B, C, and E viruses. With xenotransplantation (cross-species transfer from pigs to humans), there is a risk of transmitting porcine endogenous retroviruses (PERVs), porcine cytomegalovirus (PCMV), HEV genotype 3, porcine lymphotropic herpesviruses (PLHVs), and porcine circoviruses (PCVs). Only HEV, however, is known to infect humans in vivo, while HEV, PCV2, and PERV have been reported to infect human cells in vitro. Similarly, plant viruses are often transmitted by grafting and budding. These centuries-old techniques are used in the vegetative propagation of fruit trees and, in recent decades, vegetable crops. In grafting, the upper shoot (scion) of one plant grows on the root system (rootstock) of another plant. In the second method, a bud is taken from one plant and grown on another. Vascular continuity is eventually established, in both instances, resulting in a genetical composite that functions as a single plant. On one hand, grafting or budding onto resistant rootstocks serves as a principal tool in disease management, but on the other hand, contact between the stock to bud or scion enables the spread of viruses, even without a complete graft union. Certain parasitic plant species form connections to their hosts, similar to graft junctions, and are able to facilitate the dissemination of virus pathogens. No less than 67 plant viruses and viroids including, but not limited to CMV (Bromoviridae), TMV, Tomato mosaic virus and Tobacco rattle virus (Virgaviridae), PVY (Potyviridae), Tomato yellow leaf curl virus, and Beet curly top virus (Geminiviridae), along with Potato spindle tuber viroid (Pospiviroidae) can be transmitted between plants by at least 20 different species of the parasitic plant Cuscuta (Convolvulaceae) by means of a bridge created between infected and noninfected plant hosts. Parasitic plants produce root-like structures called haustoria which penetrate the host, connect to its vasculature and facilitate the exchange of materials such as water, nutrients, and pathogens between the host and the parasite, and between any plants simultaneously parasitized, even unrelated plant species. Cuscuta seems to act in some cases as a passive pipeline between parasitized plants as there is no replication in the parasite. Other viruses, like CMV and Grapevine leafroll-associated virus 7 (Closteroviridae), however, can replicate in Cuscuta. Additionally, Cuscuta can transmit Cuscuta-hosted viruses to the parasitized plant. Finally, parasitism by Cuscuta species presumably increases the susceptibility of plants to virus infection. Zoonosis in the Spread of Viruses

**A zoonosis refers to any disease that is naturally transmitted from animals (mostly vertebrates) to humans, or the other way around, and hence represents cross-species transmission of viruses.** A vector may be involved, or the virus is transmitted by contact with the infected host or with its direct consumption, or a derived animal product, its fluids or even a vaccine aimed at deterring infection in the intended host. As with all other pathogens, zoonotic spillovers require that the virus overcomes a hierarchical series of barriers in order to establish an infection. The probability of spillover is determined by: (1) amount of available virus (pathogen pressure), (2) dose of exposure, and (3) characteristics (genetic, physiological, and immunological) of the recipient host, that together with (2), determine the severity of the infection. Following cross-species exposure of a recipient host, the within-host barriers determine the likelihood that an infection will establish. Physical barriers include the skin, mucous membranes, mucous, stomach acidity, and absence of virus receptors. Other barriers that may block infection in both infected and neighboring cells include the innate immune response of the host.

**Vertical Transmission: Parent to Offspring Transmission of Viruses**

**Vertical transmission refers to generational transmission of viruses from parents to their offspring. HIV-1, e.g., can be acquired in utero (via breaks in the placental barrier or transcytosis of cell-associated virus), during delivery (intrapartum), or via breastfeeding.** Approximately 20% of viral plant pathogens are known to be seed transmitted. Seed transmission is commonplace in the Potyviridae. However, the mechanism by which the virus enters the seed is unknown. There is some evidence in Pea seed-borne mosaic virus (Potyviridae) that the virus may directly invade the embryo via the suspensor. On the other hand, evidence for the indirect invasion of the embryo via invasion of reproductive meristematic tissue early in plant development has been demonstrated in Barley stripe mosaic virus (Virgaviridae). At the other extreme are "vertically transmitted" viruses that live in symbiotic or commensal associations with their hosts. When temperate bacteriophages achieve a lysogenic state, they are propagated by vertical transmission to the next host generation. That is, transmission to both daughter bacteria is by cell division. Some viruses utilize both horizontal and vertical routes to transmit and maintain levels in a host population. Bee viruses are one example. Virus transmission in honey bees appears to involve foodborne transmission, venereal transmission, vector-borne transmission, and mother-to-offspring transmission. Zucchini yellow mosaic virus (Potyviridae) can also be transmitted both horizontally by aphids and vertically by seeds, but the predominant method is by horizontal transmission via aphids in a noncirculative manner. Tomato yellow leaf curl virus (Geminiviridae) is vectored between susceptible plant hosts by whiteflies (horizontal transmission); the virus is also passed from female vectors to males during copulation (also horizontal transmission) and from females via her eggs to the next generation (vertical/transovarial transmission). Although transmission of mycoviruses is typically achieved by the spread of contaminated mycelia or by hyphal anastomosis, vertical transmission also occurs through mitotic, and sometimes meiotic, spores. Very recently, however, it has been demonstrated that Sclerotinia gemycircularvirus 1 (Genomoviridae) can extracellularly infect its fungal host (Sclerotinia sclerotiorum) vectored by the mycophagous fly Lycoriella ingenua (Diptera: Sciaridae), whose progeny are also viruliferous. Most probably this is not an isolated case of a mycovirus transmitted by a vector and confirmation of more cases is pending.

**Virus Transmission by Vectors**

Besides these modes of transmission, some viruses also use a shuttle mechanism involving vectors. A vector is broadly defined as any organism, invertebrate or vertebrate, that functions as a carrier of an infectious agent between organisms. In most cases, acquisition and inoculation of the infectious agent occurs during vector feeding. Common vectors of viruses are found among the arthropods. Those with a piercing-sucking feeding behaviour such as mosquitoes (or other blood-feeding dipterans) and ticks are especially significant for vertebrate viruses, and the aphids, whiteflies, thrips, and hoppers for plant viruses. Other animal vectors of viruses include nematodes, bats, rodents, flying foxes, and horses. Species of plasmodiophorids and fungi also vector viruses. Different modes of virusvector interactions have been identified by Animal and Plant Virologists. Animal virologists recognize two major categories of virusvector relationships; viruses are said to be either mechanically transmitted or biologically transmitted by their vectors. Mechanical transmission refers to the nonspecific transmission of viruses by the vector. That is, viruses acquired externally by the vector during normal feeding behaviour on an infected organism, are inoculated during the next feed on another organism. On the other hand, biological transmission is characterized by a specific association of a virus with a particular arthropod species or genus and, more importantly, the virus is ingested by the vector and is able to propagate within the vector before transmission to another host can occur. These classifications are still in use today. Plant virologists, however, have developed a more elaborate framework to represent the types of plant virusvector interactions during transmission. Two major categories of virusvector relationships have been defined over the years that relate to the acquisition and inoculation periods, retention periods, and latent periods (the time between ingestion of the virus and the ability of the insect to inoculate a host). Namely, circulative and noncirculative. In circulative vector transmission, the virus acquired during vector feeding on an infected host, is ingested, crosses the intestinal barrier and invades the salivary glands. From there, the virus is inoculated into a new host during feeding.

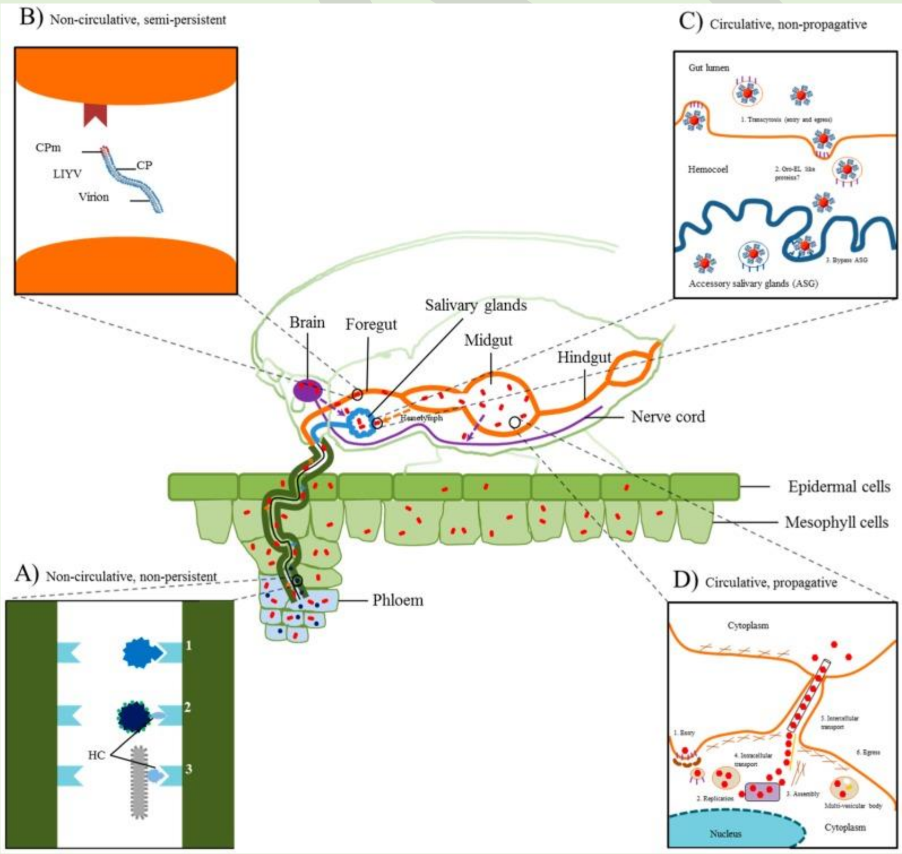
1. **Persistent mode of viral transmission**

**During the infection process of persistent, propagative viruses such as rhabdoviruses (family *Rhabdoviridae*), tospoviruses (family *Bunyaviridae*), tenuiviruses and reoviruses (family *Reoviridae*), viruses encounter multiple barriers to acquisition, replication, intercellular movement, cell escape and host plant inoculation. Virus and insect proteins thought to be involved in overcoming these barriers are only beginning to be identified.** Rhabdovirus and tospovirus surface glycoproteins are required for virus entry into vector cells (genetic determinants of transmissibility), but their counterpart receptors in the insect gut have yet to be identified. Identification of receptor-like determinants in the insect vector that viruses bind to would constitute a major breakthrough since they could become novel targets to control virus acquisition. Towards this goal, the first receptor for a circulative plant virus was recently identified in the pea aphid. Membrane alanyl aminopetidase N was shown to act as a receptor for pea enation mosaic virus (PEMV) CP in the insect gut (fig C and D).

Plant reovirus infections of their vectors lead to the generation of tubules composed of viral non-structural proteins, which interact with actin. These tubules facilitate rapid virus dissemination from the midgut across internal barriers to other insect tissues. Assembly of SRBSDV tubules that are composed of the viral non-structural protein P7-1 can be prevented by targeting the viral genome segment that encodes this protein using RNA interference (RNAi) leading to the inhibition of internal virus spread. Anti-viral RNAi technologies have great potential for plant virus control, in particular when delivered in transgenic plants.

## 2. Non-Persistent Transmission non circulative

Current evidence suggests that non-persistent plant viruses (fig A) employ one of **two mechanisms of transmission**: **capsid-only or helper-dependent**. As an example, for capsid-only mechanism, cucumber mosaic virus (CMV) particles, but not isolated viral RNA, were shown to be transmissible by the aphid *Myzus persicae*. Specifically, the viral **coat protein** (CP) and conserved capsid surface domains are required to achieve efficient aphid transmission. As an example, for helper-dependent mechanism, cauliflower mosaic virus (CaMV) was shown to require several viral proteins, along with the virions. CaMV P2 was shown to interact with the CP-anchored P3 and aphid stylet. In addition, CaMV-induced microtubule-associated transmission bodies (TBs) were shown to move key viral proteins such as P2 onto cellular microtubules upon "sensing" aphid feeding to facilitate uptake and enhance acquisition by the vector, and to target CaMV virions to microtubules for association with P2. High-resolution microscopy showed that CaMV P2 bound to aphid stylets and appeared to localize at the very tip of the stylet; this stylet region was proposed as a potential common foothold for non-circulative viruses. Potyviruses encode a helper protein, helper component-proteinase (HC-Pro), which is essential for virus transmission, as it facilitates virion retention in aphid stylets by acting as a bridge between the potyvirus CP and aphid protein(s) in the stylet. CMV 2b protein, an RNA silencing suppressor, has been shown to interfere with multiple steps of the RNA silencing pathway in plants and to indirectly enhance CMV transmission by promoting sustained phloem feeding and survival of the aphid vector.



### 3. Non-Circulative, Semi-Persistent Transmission

**Semi-persistent viruses are not thought to be internalized in the insect vector gut, but instead reside in chitin-lined areas.** Virus acquisition from the host plant and retention in the insect involve mechanisms mediated largely by the viral CP (fig B). In the case of the crinivirus, lettuce infectious yellows virus (LIYV), immune fluorescent confocal microscopy has shown that virions are retained in the foregut of the whitefly vector *Bemisia tabaci*, apparently mediated by the CP. Chen and collaborators proposed that transmission of LIYV virions from the whitefly foregut is likely to occur during regurgitation rather than salivation as seen with aphids and CaMV, given that unlike aphids, the whitefly foregut is physically separated from its maxillary stylet and salivary duct (fig B).