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The helper strategy in vector-transmission of plant viruses

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Abstract

An intriguing aspect of vector-transmission of plant viruses is the frequent involvement of a helper component (HC). HCs are virus-encoded non-structural proteins produced in infected plant cells that are mandatory for the transmission success. Over five decades, all data collected on HCs from unrelated viral species transmitted by distinct vector species were consistent with a unique mode of action designated “the bridge hypothesis”: the HC has two functional domains, one binding the virus particle and the other binding a putative receptor in the vector, creating a reversible molecular bridge between the two. This hypothesis appeared fully satisfactory as HCs were reported solely in viruses transmitted non-circulatively – i.e. the virus particle binds externally to the mouthpart of its vector, and can later be released therefrom and inoculated. Recently, however, HCs have also been reported in viruses transmitted circulatively, where the virus particles are internalized in gut cells and cycle within the body to reach the salivary glands. In this more complex scheme of virus-vector interaction, a simple mode of action of HC compatible with the bridge hypothesis becomes questionable. In addition, while it had consistently been shown that the sequential acquisition of HC and virus particles could only work when HC was acquired first, a recent report shows that the reverse acquisition sequence can work in some case, again questioning the bridge hypothesis as a universal mode of action. Because of the importance of HC molecules in the vector-transmission of plant viruses, we here propose an exhaustive review of the field, of its historical perspective and most recent development.

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1. Introduction

Vector transmission is the mean by which most plant viruses (around 80%) ensure plant-to-plant transfer, and thus maintenance and spread in the environment. Any organism feeding on plants and capable of moving from one plant to another can uptake and release viruses and can potentially act as a vector. Diverse organisms (protists, nematodes, mites and insects) have been reported as efficient vectors, but sap feeding hemipteran insects encompassing planthoppers, leafhoppers, and particularly whiteflies and aphids are by far the most important (Nault, 1997; Ng & Falk, 2006; Hogenhout et al., 2008). The mechanisms of virus-vector interaction are also diverse and have been extensively studied in hemipteran insects, which are the major focus of this review. Nevertheless, the classification of the transmission modes of hemipteran insect-transmitted viruses can accommodate cases of transmission by all types of vectors, including mites, nematodes and protists (Blanc, 2008).

The two major transmission categories are named non-circulative and circulative (Blanc et al., 2014). In non-circulative transmission, the plant-vector interaction is exclusively external. In hemipteran vectors, the virus reversibly binds to the mouthparts and/or the foregut, where it can be retained for a short time (few minutes to hours) and immediately inoculated to another plant upon salivation or regurgitation. In contrast, in circulative transmission, the interaction is internal. In hemipteran vectors, this means that the virus crosses the gut and circulates within the insect body to reach the salivary glands and ultimately the saliva. Whatever the details of this cycle, it involves an initial specific recognition and the crossing of one or several cellular barriers (at least gut and salivary glands) to accumulate within and be secreted out of the vector. The consequences are the existence of a latent period between acquisition and inoculation, and also, in all reported cases, a high and long-lasting accumulation of the virus that can be transmitted for the whole vector's life span (Blanc et al., 2014). The circulative transmission is divided in the propagative and non-propagative sub-categories, depending on whether the virus replicates or not during its cycle within the vector (Blanc & Gutiérrez, 2015).

Regarding molecular interactions with the vector, plant viruses have evolved two distinct strategies, the "capsid strategy" and the "helper strategy" (Pirone & Blanc, 1996). In the capsid strategy, the virus particles are necessary and sufficient to ensure the specific recognition of the vector. The experimental demonstration came from the capacity of the corresponding viruses to be directly acquired from suspensions of purified virus particles, without any other viral proteins, and later transmitted to healthy host plants (Pirone, 1964), demonstrating that the coat protein alone can control attachment (and cycle) within the vector. However, all viruses are not transmissible this way. In addition to the virus particle, a virus-encoded non-structural protein is mandatory for the helper strategy. Many viral species have been shown to require such additional proteins that have been designated "helper components" (HC). Since the first case report for a species of the genus *Potyvirus* (Kassanis & Govier, 1971a), HCs have been described in many other non-circulative virus genera and the helper strategy proved dominant in this transmission category (Syller, 2006). Because HCs of unrelated viruses have no sequence homologies, a specific definition has been provided (Froissart et al., 2002): "a HC can be any compound complying with all of the following criteria: (i) it is virus-encoded, (ii) it can be one or a complex of several non-structural molecules (*i.e.* which can be eliminated upon purification of virions), (iii) it is necessary for the success of vector transmission, and (iv) HC and virions can be acquired sequentially by the vector".

Until today, the HCs from unrelated plant viruses were thought to have the same mode of action, illustrating a convergent evolution, although the selective advantage they may provide remains speculative (Pirone & Blanc, 1996; Froissart et al., 2002). One unexplained longstanding observation was the fact that the helper strategy appears frequent in non-circulative transmission but extremely rare in circulative transmission (Franz et al., 1999). Recent advances are challenging these views by showing that the helper strategy appears widespread in all transmission categories (Grigoras et al., 2018; Lu et al., 2019; Di Mattia et al., 2020), and that distinct HCs may have different modes of action (Di Mattia et al., 2022). In this review, we confront the historical context and the recent discoveries, and we discuss how this wealth of knowledge indicates that HCs may have evolved for diverse reasons in distinct viral clades.

2. A historical perspective on the discovery of helper components

The discovery of HCs and the characterization of their mode of action results exclusively from studies of non-circulatively transmitted viruses. Indeed, as already mentioned above, that the helper strategy also exists in circulative viruses is a very recent finding; it is poorly characterized and will be discussed in a later specific section.

The first hint on the discovery of HCs came from (Pirone & Megahed, 1966) who observed that purified particles of the cauliflower mosaic virus (CaMV, genus *Caulimovirus*, Family *Caulimoviridae*) or of the turnip mosaic virus (TuMV, genus *Potyvirus*, family *Potyviridae*) were not transmissible, when acquired through parafilm membranes by aphid vectors. At that time the explanation was unclear and could as well be attributed to partial degradation of virions during the purification process. However, about five years later, key complementary information was reported on the same viral genera. In a series of experiments based on sequential acquisition of transmissible and non-transmissible natural isolates, Kassanis & Govier, and in parallel Lung and Pirone, respectively set the basis of the concept of HC for potyviruses (potato virus Y, PVY, genus *Potyvirus*) and caulimoviruses (CaMV) (**Table 1**). These authors first demonstrated that a non-transmissible isolate could be efficiently transmitted by aphids previously fed on a plant infected with a transmissible isolate of the same viral species, and that the reverse acquisition sequence did not work (Kassanis & Govier, 1971a; b; Lung & Pirone, 1973). These experiments pointed at the existence of a helper factor that would be produced in the plants infected by the transmissible isolate. Such a helper factor could be acquired first by aphids and somewhat be retained and "wait" somewhere in the alimentary tract to complement the transmission of the secondarily acquired non-transmissible isolate. The same authors consistently showed that the transmission of purified virus particles of both poty- and caulimoviruses could be rescued by pre-feeding aphids onto plants infected with a transmissible isolate (Govier & Kassanis, 1974; Lung & Pirone, 1974). Potyvirus-infected plant extracts deprived of virus particles by ultracentrifugation contained HC activity, indicating that the HC is not the virus particle of the transmissible isolate itself (Govier & Kassanis, 1974). Additional chemical, biochemical and immunological treatments of potyvirus-infected plant extracts further demonstrated that the HC is a non-structural protein produced upon infection of plants by a transmissible isolate (Govier et al., 1977). Antisera directed against viral proteins produced from cell-free translation of viral RNA provided the first hint that the HC is virus-encoded by specifically blocking its activity in infected plant-extracts (Hellmann et al., 1983; Thornbury & Pirone, 1983). The copurification of potyviral HC activity and of a viral polypeptide of around 50 KDa further pointed in the same direction (Thornbury et al., 1985). Nevertheless, it was not before the development of molecular biology, particularly the sequencing technology and the production of infectious clones allowing reverse genetic approaches, that the viral origin of HCs could be directly proven.

Potyvirus

The genome of potyviruses is a ss(+)RNA encoding one single large polyprotein precursor, post-translationally cleaved in ten polypeptides, each with one or more specific functions (Allison et al., 1985; Domier et al., 1986; Dougherty & Carrington, 1988). The polypeptide co-purifying with the HC activity (Thornbury et al., 1985) was identified as the second from the N-terminus of the polyprotein (Carrington et al., 1989). Because it also possesses a protease activity, releasing it from the large precursor polyprotein, it was named the helper component proteinase (HC-Pro) (Carrington et al., 1989). Comparison of HC-Pro sequences from transmissible and non-transmissible potyvirus species and isolates pointed at differences in two key regions. The first is located in the N-terminal region (AA positions 50-54) where a conserved "KITC" motif is mutated to EITC in non-transmissible isolates (Thornbury et al., 1990; Granier et al., 1993; Canto et al., 1995). The second is located in the central region (AA positions 308-310) where a conserved "PTK" motif is mutated to PAK in non-transmissible isolates (Huet et al., 1994). The key role of both motifs in HC activity has been thoroughly confirmed by reverse genetics where the introduction of these mutations in transmissible infectious clones always abolished their capacity to be transmitted by aphids (Atreya et al., 1992; Atreya & Pirone, 1993; Huet et al., 1994).

Table 1 - List of viral families and genera where HCs have been identified

Virus family[a]	Virus genus[a]	Virus name/acronym[a]	HC name/acronym	Transmission mode	Vector taxon	First HC-describing reference
Potyviridae	*Potyvirus*	Potato virus Y (PVY)	Helper-component proteinase (HC-Pro)	Non-circulative	Aphids	Kassanis and Govier 1971a,b
	Tritimovirus	Sweet potato mild mottle virus (SPMMV)			Whiteflies	Colinet et al 1998
	Ipomovirus	Wheat streak mosaic virus (WSMV)			Mites	Stenger et al 2005
Caulimoviridae	*Caulimovirus*	Cauliflower mosaic virus (CaMV)	Aphid transmission factor (P2)		Aphids	Lung and Pirone 1973
Virgaviridae	*Tobravirus*	Tobacco rattle virus (TBRV)	Protein 2b		Nematodes	McFarlane et al 1996
Phenuiviridae	*Tenuivirus*	Rice stripe virus (RSV)	Non-structural glycoprotein 2 (NSvc2)	Circulative propagative	Planthoppers	Lu et al 2019
Nanoviridae	*Babuvirus*	Faba bean necrotic yellows virus (FBNSV)	Nuclear shuttle protein (NSP)	Circulative non-propagative	Aphids	Franz et al 1999
	Nanovirus[b]					

[a] Families and genera listed in this table are only those where the HC has been identified. The existence of an HC has been suggested in others but not identified.

[b] Babuviruses have a DNA segment highly homologous to the segment N (encoding NSP) of nanoviruses and so its role as a HC makes little doubt though the lack of infectious clones has precluded definitive proof.

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Caulimovirus

The comparison of the sequence of distinct CaMV isolates revealed that the non-transmissible CM4-184 isolate harbors a 421 bp deletion in the coding region II (gene II) (Howell & Hull, 1978; Howarth et al., 1981). These authors thereby established that gene II was dispensable for plant infection, pointing at its specific function in aphid-transmission. This was soon confirmed by the demonstration that deletion or mutation of this genome region in transmissible CaMV isolates were associated with the loss of transmissibility (Armour et al., 1983; Daubert et al., 1983; Woolston et al., 1983, 1987). The product of gene II, the protein P2, has then been produced in the heterologous baculovirus/insect cell system (Espinoza et al., 1992), in a biologically active form able to complement the transmission of CaMV non-transmissible isolates acquired from infected plants or crude extracts thereof (Blanc et al., 1993). Intriguingly, this heterologous-expressed P2 failed to complement the transmission of purified CaMV particle, suggesting the possible requirement for an additional unknown factor (Blanc et al., 1993). Finally, this additional compound was identified as the viral protein P3 (product of gene III) that must be present in the purified CaMV particle suspension for successful complementation of aphid-transmission by the previously acquired P2 (Leh et al., 1999). Thus, two non-structural proteins, P2 and P3, assist the vector-transmission of CaMV. While P2 meets the four criteria defining a HC (Froissart et al., 2002), P3 does not. Indeed, its acquisition together with CaMV virions is mandatory for successful vector-transmission (Drucker et al., 2002). As P3 cannot be acquired separately from virions, it cannot be qualified as a HC.

While the helper strategy has been extensively studied for the genera *Caulimovirus* and *Potyvirus*, it has also been reported in other genera of the family *Potyviridae*: *Ipomovirus* with viruses transmitted by whiteflies (Colinet et al., 1998) and *Tritimovirus* with viruses transmitted by mites (Stenger et al., 2005; Stenger et al., 2005; Stenger et al., 2006) (**Table 1**). The requirement of HC molecules has even been evidenced in the genus *Tobravirus* (Family *Virgaviridae*) transmitted by nematodes in a non-circulative manner (MacFarlane et al., 1999) (**Table 1**). Hence, helper components appear to have evolved in unrelated viruses transmitted non-circulatively by distinct vectors. Unfortunately, in depth characterization of their mode of action remain limited to a few emblematic cases within the poty- and caulimoviruses.

3. Modes of action of helper components

The repeated observation that successful transmission resulted either from the concomitant acquisition of the HC and virus particle, or from their sequential acquisition solely if the HC is acquired first, allowed the pioneer research groups working on potyviruses (Kassanis & Govier, 1971b; a) and caulimoviruses (Lung & Pirone, 1973, 1974) to propose an hypothesis describing the mode of action of HCs. The HC would specifically bind to a receptor molecule within the vector and to the virus particle (or to a viral partner fixed on the virus particle in the case of CaMV), through distinct functional domains, thereby creating a molecular link between the two. This hypothesis, later named the “bridge hypothesis” (Pirone & Blanc, 1996) became widely accepted and, so far, nearly all empirical investigations on the question yielded supportive results. Remarkably, recent data on the existence of HCs in circulative viral species is casting doubts or at least calling for nuances and careful reexamination (see the dedicated section IV).

3.1 Helper component – virus particle interaction

Potyvirus

As indicated in the previous section, reverse genetic approaches identified two important domains of the potyviral HC-Pro, respectively containing the motifs KITC and PTK. In parallel, comparison of coat protein sequences pointed out the highly conserved amino acid triplet DAG that appears to be mutated to DAE in some of the analyzed non transmissible isolates (Harrison & Robinson, 1988). Directed mutagenesis in infectious clones of various potyviral species, changing the triplet DAG to DAE, systematically resulted in the loss of aphid-transmissibility (Atreya et al., 1990, 1995; Gal-On et al., 1992). Noticeably, the DAG motif is located at the N-terminus of the coat protein, in a region predicted to be accessible at the surface of virus particle, potentially available for binding to HC-Pro (Shukla & Ward, 1989). The experimental demonstration of a direct interaction between HC-Pro and the capsid of Tobacco vein mottling virus (TVMV) was obtained using a protein blotting-overlay protocol where the HC-Pro extracted from infected plants was assessed for specific binding onto full length or truncated coat protein. HC-Pro was shown to

interact with a 7 amino acid sequence (DTVDAGK) encompassing the DAG motif, and this interaction was totally abolished by the DAE mutation. Several amino acid substitutions were performed within the DTVDAGK sequence and used to demonstrate a perfect correlation between HC-Pro/coat protein binding and successful aphid transmission (Blanc et al., 1997) (Figure 1). The HC-Pro-CP interaction was then reported for other potyviruses (Peng et al., 1998) and the PTK amino acid triplet identified as the HC-Pro motif recognizing the DAG of the coat protein (Peng et al., 1998) (Figure 1). Further experimental evidence came from a study on the specificity of the binding between HC-Pro and CP. The HC-Pro of zucchini yellow mosaic virus (ZYMV) cannot complement the transmission of TuMV particles, and *vice versa*. Replacing the N-terminal region of the coat protein of ZYMV by that of TuMV allowed the aphid transmission of the recombinant virus complemented by the HC-Pro of TuMV (Wang et al., 1998). This result strongly suggested that the N-terminal region of the CP containing the DAG motif is the sole determinant of the binding of potyvirus particles to HC-Pro (Figure 1).

Caulimovirus

Protein overlay assays demonstrated a more complex situation for CaMV (Schmidt et al., 1994). The HC (the protein P2) efficiently attached virions from infected plant crude extracts but not from purified virus suspensions. The mandatory additional component eliminated upon purification was later found to be the viral protein P3 (Leh et al., 1999, 2001; Drucker et al., 2002), that decorates virus particles, with its C-terminus deeply anchored into pores opened in between capsid hexamers, and its N-terminus exposed at the surface and binding to P2 (Plisson et al., 2005; Hoh et al., 2010) (Figure 1). It is remarkable that P3 adopts a tetrameric parallel conformation when free in solution and a different one when anchored to the virion, and that only the latter conformation has an affinity for a functional multimeric form of P2 (Hebrard et al., 2001; Hoh et al., 2010). These studies further determined that the virion-bound P3 forms a network of N-terminal antiparallel coiled-coil dimers that bind to a coiled coil trimer of the C-terminal moiety of P2 (Figure 1).

3.2 Helper component – aphid receptor interaction

Potyvirus

In the case of potyviruses, seminal work by Bradley and Ganong (Bradley & Ganong, 1955a; b) showed that irradiation or chemical treatment with formaldehyde of the stylet's tip of viruliferous aphids inhibited subsequent inoculation of PVY, suggesting that the infectious virus material was retained at the corresponding location. Consistently, autoradiography of labeled virions as well as optical, electron, and more recently fluorescent microscopy, showed that potyviruses can be detected throughout the alimentary tract, but that they are mainly retained at the distal tip of the aphid stylets (Ammar et al., 1994; Wang et al., 1996; Mondal et al., 2021). Transmission assays with various potyviruses and aphid species demonstrated that the transmission success depends largely on the HC-Pro/aphid species combination, and this was considered a first indication of the probable existence of specific receptors of HC-Pro within aphid stylets (Wang et al., 1998).

We here again refer to the two conserved amino acid motifs, KITC and PTK, respectively located in the N-terminal and central regions of HC-Pro. While, as indicated above, mutations in the PTK motif abolish the binding to the coat protein (Huet et al., 1994), changes in KITC motif (and even in a larger N-terminal domain) do not but instead impair retention within the stylets (Blanc et al., 1998) (Figure 1). Biochemical and structural analysis of HC-Pro revealed its capacity to self-interact and form oligomers (Thornbury et al., 1985; Plisson et al., 2003; Ruiz-Ferrer et al., 2005), and Ruiz-Ferrer and colleagues (2005) further speculated that this oligomerization drives a geometrical arrangement and generates two functional domains, which could respectively bind to virion and to a receptor in the stylets.

An elegant study, combining the electrical control of aphid feeding behavior and transmission testing, demonstrated that the acquisition of a potyvirus is associated to ingestion of infected plant cell content, whereas the inoculation is associated to salivation (Powell, 2005). The food canal where the sap is streaming up and the salivary canal where the saliva is streaming down are separated all along the stylets, but at the extreme distal tip where they fuse to form the "common canal". The authors logically concluded that the HC-Pro-virion complexes should be retained in the common canal, because it is the only location in contact with both ingested plant material and ejected saliva. Despite the fact that all available

experimental data are compatible with the bridge hypothesis (Dombrovsky et al., 2007; Fernandez-Calvino et al., 2010; Kamangar et al., 2019), the identity of the receptor of HC-Pro within the common canal remains elusive. Stylets being cuticular tissues, cuticular proteins are of course expected to play a role in potyvirus binding and represent the best receptor candidates, but the proof is still lacking.

Caulimovirus

Just as for potyviruses, the domain of the CaMV P2 that recognizes the putative receptor within the aphid stylets has been localized by mutagenesis approaches in the N-terminal domain of the protein. In particular, the identity of the residue at amino acid position 6 appears to be key for aphid/virus recognition. In the CaMV Cabb-BJI isolate, replacement of the glutamine at this position by other residues differentially affected the transmission efficiency by distinct aphid species. One of these mutants, where a tyrosine substituted for the glutamine, named mutant P2-Rev5, could no longer be transmitted by any aphid species (Moreno et al., 2005).

A major advance in this field of research came from the development of an efficient in vitro binding assay between the heterologously-expressed CaMV P2 and dissected aphid maxillary stylets (Uzest et al., 2007)**.** This system confirmed that the P2-Rev5 mutant does not bind to the stylets whereas the wild type P2 is specifically retained within the common canal, onto an unforeseen cuticular structure that has been named the “acrostyle” (Uzest et al., 2010) (Figure 1). Initially reported as displaying cuticular proteins at its surface, the acrostyle has been further characterized and its proteome is now available (Webster et al., 2017, 2018; Deshoux et al., 2020). Of important note is the identification of proteins from the CPR and CPAP3 families, named Stylin-01 to Stylin-05, that have one of their domains emerging and accessible at the surface of the acrostyle (Figure 1). All of these proteins stand as prime receptor candidates for non-circulative viruses and, consistently, Stylin-01 was shown to be involved in CaMV transmission and has been proposed to act as receptor for this virus (Webster et al., 2018) (Figure 1). Unfortunately, cuticular proteins are extremely difficult to handle. Their biochemical and structural properties are virtually unknown, and further effort is needed to achieve heterologous production in a correctly folded and fully functional form. This technical bottleneck has been holding for decades and has thus far precluded the definitive validation of HC–receptor interaction. Breaking this lock would pave the way for the search of receptors of potyviruses and/or cucumoviruses, potentially among the Stylins described in the acrostyle, and for the development of antagonistic compounds able to specifically block virus-vector interactions.

As indicated earlier, studies of the HC mode of action in non-circulative virus clades other than caulimo- and potyviruses are scarce. To briefly mention in the frame of this review, the reports on HC-Pro in distinct genera of the family *Potyviridae*, transmitted by whiteflies or even mites, all assume a mode of action similar to that in the genus *Potyvirus* transmitted by aphids (Colinet et al., 1998; Stenger et al., 2005). Likewise, investigation of the mode of action of the HC in tobraviruses indicated that virus particles can be retained in the anterior part of the feeding apparatus of nematode vectors only when a compatible HC is also present and associated to virions, again supporting the bridge hypothesis (MacFarlane et al., 1999; Vassilakos et al., 2001).

3.3 Transmission activation

At least the two best-studied groups of non-circulative viruses, potyviruses and caulimoviruses, have evolved a remarkably sophisticated cellular response to the feeding of the insect vectors on infected plants. This response results in the immediate formation of specific viral transmission morphs that are efficiently acquired by the vectors. In both cases, the helper component is a key player of this phenomenon that has been called “Transmission Activation” or TA (Drucker & Then, 2015).

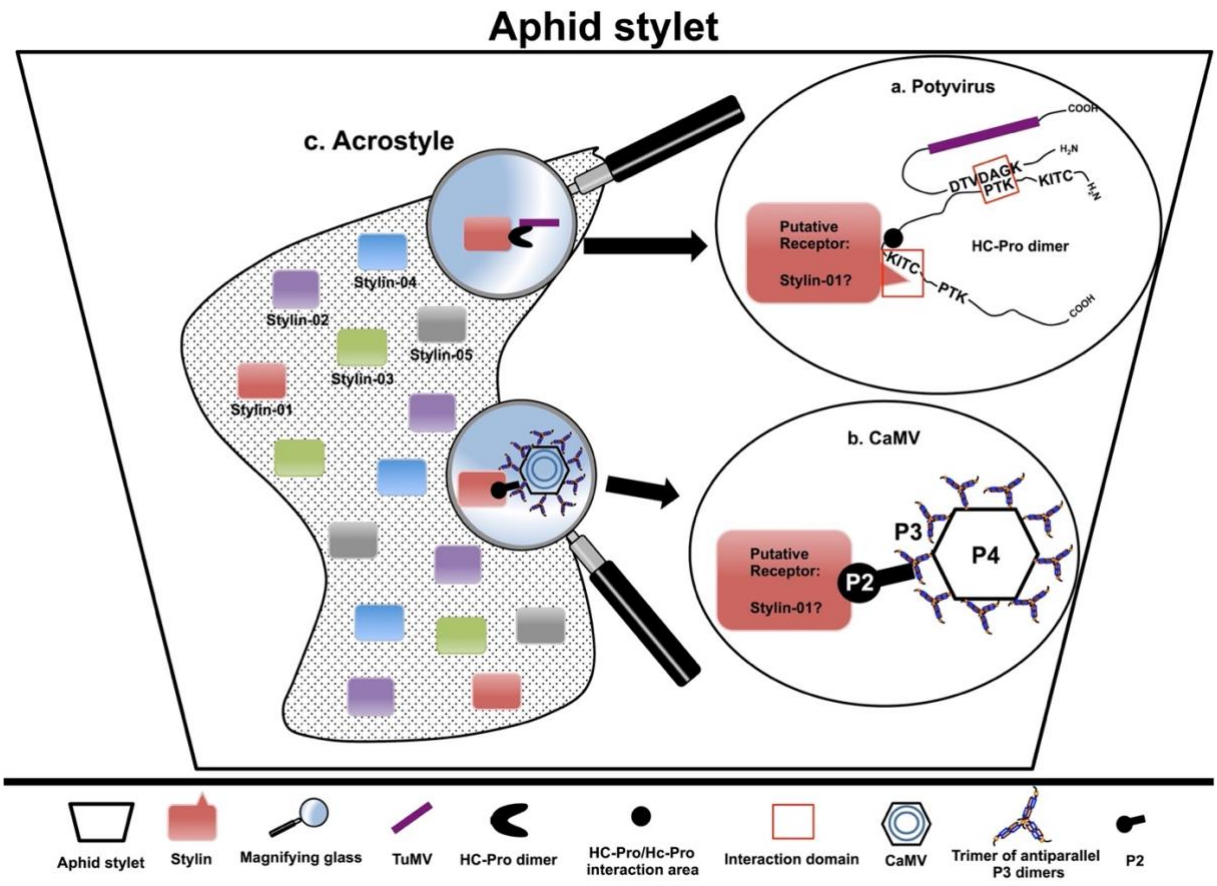

Figure 1 - Schematization of the HC mode of action for noncirculative viruses transmitted by aphids. (a) Potyvirus: shows Hc-Pro attached to putative receptors. (b) CaMV: shows the HC (P2) bound to putative receptor; P4 is the coat protein; P3 forms a network around the virus particle. At the distal tip of the aphid stylets, the acrostyle (c) harbors cuticular proteins named Stylins (Stylin-01 to -05). All known or suspected stylet-HC-virus particle interactions and the involved protein domains (when identified) are detailed in the maginifications on the right

Within infected plant cells, the three components of the CaMV transmissible complexes, P2, P3 and virions, are separated in distinct compartments. Most of the virus particles are accumulated, likely as P3-virion complexes, in several electron-dense inclusions that have been identified as the viral factories. P2 is not detectable in these viral factories. Instead, P2 entirely accumulates in one unique electron-lucent inclusion per infected cell, that also contain P3 and a few scattered virions (Drucker et al., 2002), and that is designated the transmission body (TB). A series of studies uncovered a phenomenon where the CaMV can reversibly produce transmissible complexes, precisely when needed, when aphids puncture plant cells and ingest a minute amount of their content (Martinière et al., 2009; Martiniere et al., 2013; Bak et al., 2013). Upon puncturing an infected plant, the stylets and/or secreted saliva trigger a plant response, likely involving calcium fluctuation and ROS production, which is immediately hijacked by the virus. Within less than ten seconds, the TB is first loaded with tubulin and then disintegrates and disperses P2 all over the cell cytoplasm onto the microtubule network (Martiniere et al., 2013). Within the same short time frame, P3-virion complexes are expulsed from the viral factories and also totally cover the microtubule network, likely associating with P2 (Bak et al., 2013). The authors showed that transmissible complexes thereby become accessible all over the cell probed by an aphid vector, and further demonstrated that the situation reversed to the initial stage within 5 minutes of aphid departure, with the reconstitution of genuine TB (Martiniere et al., 2013) and virion-loaded viral factories (Bak et al., 2013).

A comparable observation has been reported by the same research group for potyviruses (Berthelot et al., 2019). While the phenomenon of transmission activation can be considered analogous, the molecular/cellular processes involved are totally distinct (Berthelot et al., 2019). In this case, both the virus particles of TuMV and the HC-Pro are homogeneously distributed over the cytoplasm of infected plant

cells. The signal of the aphid puncture and its transduction by the plant, possibly also involving calcium spiking and ROS production, induces an immediate oligomerization of HC-Pro, and this conformational change appears to promote the formation of HC-Pro-virion complexes within the cell. The same study again demonstrates that this phenomenon is reversible after a few minutes from the triggering signal, with disappearance of the oligomeric forms of HC-Pro within infected tissues.

In the two cases, one may wonder why a virus would adopt such a complex "behavior" when a constitutive production of transmissible complexes in infected tissues would appear easier and efficient. The explanation provided by the authors is that viral proteins are often multifunctional and have many duties to fulfill. For example, CaMV-P3 complexes also bind to P1 for cell-to-cell and long-distance movement within the plant (Stavolone et al., 2005) and the domain of P3 involved in binding to P1 is the same as that binding to P2. The HC-Pro of potyviruses has multiple functions (Valli et al., 2018), many of which may not be compatible with the oligomeric form binding to the virus particles upon aphid punctures. In simple words, the transmissible complexes may interfere with other step of the viral life cycle and their transient appearance just when needed may alleviate the problem.

Whether TA is a general phenomenon that could be extended to the transmission of many other viral species is a formidable avenue of research in the near future, even beyond plant virology (Blanc & Gutiérrez, 2015). Likewise, whether it is limited to non-circulative transmission and more specifically whether HC molecules should always be involved is also a relevant and stimulating question.

4. The helper strategy in circulative viruses

The helper strategy has long been believed to be restricted to the non-circulative transmission, though no explanation could be provided. There was one report, mentioning the involvement of an HC in the aphid transmission of a nanovirus (Genus *Nanovirus*, Family *Nanoviridae*) (Franz et al., 1999), but the molecular details were not investigated for nearly two decades. This uncharacterized HC controlling the transmission of a circulative non-propagative nanovirus remained the exception confirming the rule until recently. The discovery of the involvement of a HC in the planthopper transmission of the circulative propagative rice stripe virus (RSV, genus *Tenuivirus* Family *Phenuiviridae,* Order *Bunyavirales*) confirmed that a helper strategy can evolve whatever the category of virus-vector interaction (Lu et al., 2019) (Table 1). An interesting and timely question is now whether the bridge hypothesis stands as the universal mode of action of HCs.

Tenuiviruses

Most members of the order *Bunyavirales* have a lipid envelope associated with two glycoproteins Gn and Gc involved in membrane fusion and entry into host cells (Guardado-Calvo & Rey, 2017). Tenuiviruses appear to have lost their lipid envelop but still produce the two glycoproteins, which are in this case non-structural proteins respectively named NSvc2-N and NSvc2-C (Chen et al., 2019), deriving from the cleavage of the precursor glycoprotein NSvc2. The non-enveloped virus particles appear as filamentous ribonucleoproteins (Toriyama, 1986) and, interestingly, they cannot be transmitted by their planthopper vectors when purified (Lu et al., 2019). The adjunction of the two glycoproteins to purified ribonulceoproteins efficiently rescued the vector transmission of rice stripe virus (Lu et al., 2019). The same authors further demonstrated that the two proteins can interact with the virus particle. NSvc2-N connects it to a putative receptor at the surface of the insect gut cells and triggers endocytosis. NSvc2-C subsequently allows the release of the virion-NSvc2-N complex from endosomes into the cytosol, presumably through a membrane fusion activity whose precise mode of action remains elusive in the specific case of tenuiviruses (Figure 2a).

These results are seemingly compatible with the bridge hypothesis, at least for NSvc2-N (Yao et al., 2014), but some aspects need further investigations. In particular, it is unclear whether NSvc2-C can be acquired sequentially or whether a co-acquisition with the virion and/or NSvc2-N is mandatory. Because Gn and Gc of bunyaviruses are generally forming heterodimers (Hepojoki et al., 2010), it would also be interesting to test a possible co-acquisition of NSvc2-N and NSvc2-C as a unique complex that could complement the ulterior acquisition and transmission of purified viral particles.

Nanoviruses

Analogous to approaches earlier developed for non-circulative poty- or caulimoviruses (see section 2), Franz and coworkers discovered that the aphid transmission of purified faba bean necrotic yellows virus (FBNYV, genus *Nanovirus*) particles was not possible, unless the aphid vectors were previously fed on a plant infected by a transmissible isolate (Franz et al., 1999) (Table 1). Similar pre-feeding on an infected plant was also required for the transmission of purified viral particles directly injected into the aphid hemolymph. Although its viral origin was not proven, the authors concluded that a HC was most likely produced in infected plants and necessary for the virus to cross the gut and salivary gland cell barriers. Unfortunately, both the identity and the mode of action of this putative HC remained unexplored after this seminal work, even long after infectious clones of nanoviruses became available.

The family *Nanoviridae* regroups viruses which genome is composed of six (genus *Babuvirus*) or eight (genus *Nanovirus*) circular ssDNA segments, individually encapsidated in distinct viral particles and each encoding a single protein (Gronenborn, 2004; Lal et al., 2020). Infectious clones could be successfully produced for several member species of the genus *Nanovirus* (Timchenko et al., 2006; Grigoras et al., 2009, 2014, 2018), but so far failed for babuviruses. Because of the nature of these infectious constructs, one clone per genomic segment, these authors realized that some segments could be omitted upon inoculation without compromising the systemic infection of host plants (Timchenko et al., 2006; Grigoras et al., 2018). They were then able to demonstrate that in the absence of the segment N, the host plant appeared systemically infected but aphid transmission from this plant was impossible (Grigoras et al., 2018). Modifications of the start codon or coding sequence further showed that the HC of nanoviruses is not the segment N itself but its encoded NSP protein (Grigoras et al., 2018; Di Mattia et al., 2020).

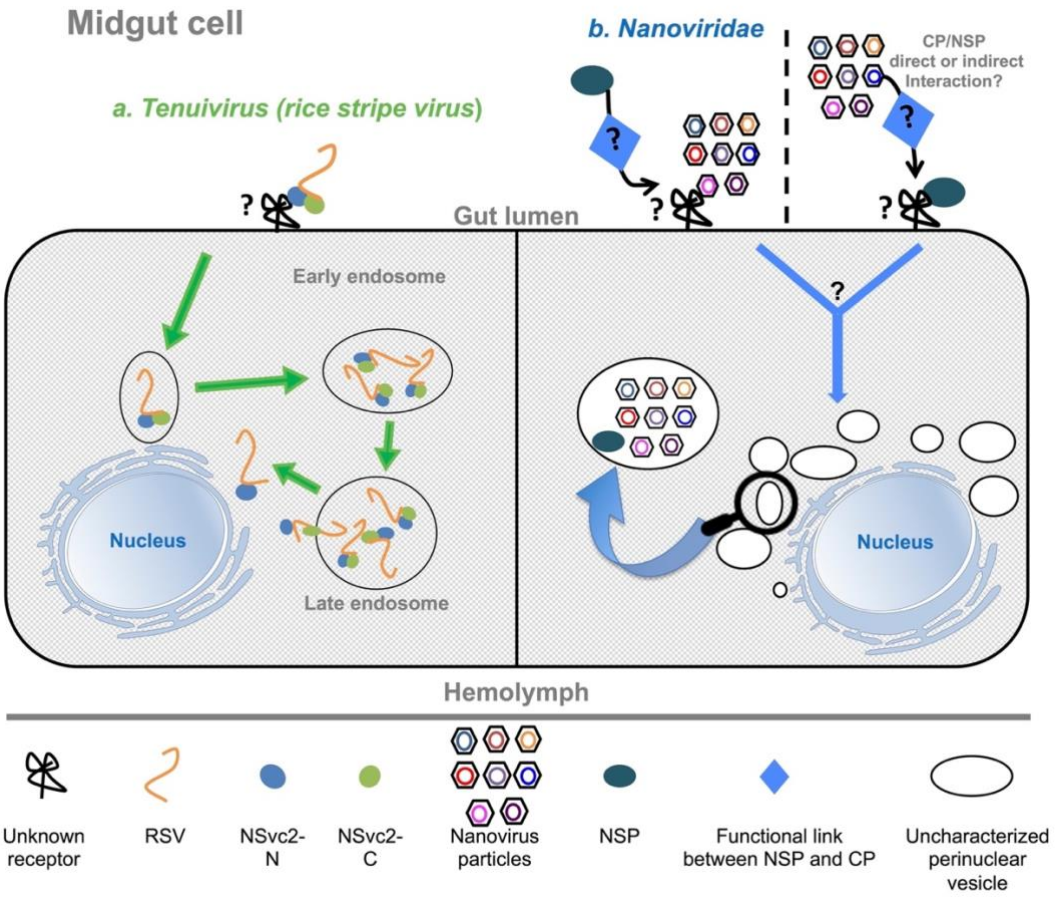

Figure 2 - Schematization of the hypothetical mode of action of HC for circulative viruses transmitted by hemipteran insects. The mode of action of HC in circulative propagative transmission of tenuiviruses is summarized on the left. The mode of action of the HC of circulative non propagative nanoviruses is shown on the right. There is a clear lack of molecular details that are currently awaiting further investigation.

The localization of the coat protein of banana bunchy top babuvirus in its aphid vector indicated that the virus accumulates at only two locations, the anterior midgut and the principal salivary glands (Bressan & Watanabe, 2011; Watanabe & Bressan, 2013). Di Mattia and co-workers (Di Mattia et al., 2020) recently re-investigated this localization for faba bean necrotic stunt virus (FBNSV, genus *Nanovirus*), in the presence/absence of the segment N. Not only did they observe that this segment was mandatory for the internalization of the virus and its accumulation in the aphid gut cells, but also that the NSP protein colocalized therein with both viral DNA and coat protein. This observation is reminiscent of that described for NScv2-N of tenuiviruses (Lu et al., 2019) and again compatible with the bridge hypothesis. The NSP protein may attach to the viral particles on one side and to a gut (and/or salivary gland) cell receptor on the other side and trigger the internalization of the complex (Di Mattia et al., 2020). However, most recent incongruent observations are now casting doubts (Figure 2b).

A thorough characterization of protein-protein interactions was conducted between the eight proteins of another nanovirus, the pea necrotic yellow dwarf virus (PNYDV), using bimolecular fluorescence complementation (BiFC) (Krenz et al., 2017). This approach revealed that NSP interacts with itself and with M-Rep (the replication-associated protein) but, surprisingly, not with the coat protein CP. Because an interaction between NSP and CP has since then been reported for babuviruses (Ji et al., 2019; Yu et al., 2019), this question deserves further investigation for nanoviruses and the two possibility – direct NSP/CP interaction or not – are envisaged in Figure 2b. In the BiFC system, the fusion of fluorescent reporter protein to NSP protein of FBNSV may have hindered the interaction with CP.

One highly consistent observation on the action of HCs in non-circulative transmission is that it must be acquired first, prior to the virus particles, the reverse acquisition sequence being systematically inefficient. This has always been interpreted as related to the capacity of the helper to recognize its receptor in the vector mouthparts or foregut, while the virus particle cannot directly do this and is "flushed" with the digestive flux/transit when acquired first and alone. Recent work from our laboratory yielded a contradicting observation (Di Mattia et al., 2022). As mentioned above, some genome segments of nanoviruses can be omitted at inoculation of host plants and we made use of this possibility to design sequential acquisition experiments. Plants infected with either the segment N or the segment U4 missing (respectively named FBNSV-N$^{(-)}$ and FBNSV-U4$^{(-)}$) exhibit symptoms similar to those resulting from wild type FBNSV infection, and FBNSV-U4$^{(-)}$ is perfectly transmissible by aphid while FBNSV-N$^{(-)}$ is not (Grigoras et al., 2018). In fact, U4 is completely dispensable for infection and aphid transmission under laboratory conditions. According to the current understanding of the mode of action of HCs, aphids fed first on the FBNSV-U4$^{(-)}$ infected plants should acquire NSP and subsequently complement the acquisition and transmission of the segment U4 from FBNSV-N$^{(-)}$ infected plants. In contrast, in the reverse acquisition sequence, U4-containing virus particles should not be internalized in and transmitted by aphid, due to the absence of NSP protein in the first acquisition from FBNSV-N$^{(-)}$ plants, and due to their absence in the second acquisition from FBNSV-U4$^{(-)}$ plants. To our surprise, U4 segment was as efficiently internalized in and transmitted by aphids whatever the sequence of acquisition (Di Mattia et al., 2022). This proved true even when aphids were "purged" on healthy plants for up to 2 days in between the two acquisition phases. This observation is intriguing and is further discussed in the last section. It demonstrates that, in the case of nanoviruses, the HC can expectedly wait for virions within the aphid, but that the virions can somewhat unexpectedly wait for the HC for at least two days without being flushed out by the digestive flux.

5. New perspectives and prospects

5.1 What is a helper component?

When Froissart and colleagues (Froissart et al., 2002) proposed the extended definition of HC, the helper-dependent strategy was almost exclusively described for non-circulative viruses. Most knowledge on the nature and mode of action of HCs were based on the thorough characterization of the best-studied cases, P2 of CaMV and HC-Pro of potyviruses, but the proposed definition also accounted for the fragmentary data on HCs of other virus groups available at that time (Table 1). Since then, additional reports have been published, the most intriguing being those confirming the existence of the helper strategy in circulative transmission (Grigoras et al., 2018; Lu et al., 2019; Di Mattia et al., 2020, 2022). In all cases, HCs are non-structural proteins encoded by the virus and they are mandatory for the success of vectors transmission. One key feature of HCs that is confronted by these recent findings is the sequential

acquisition of HC and virions. While the sequential acquisition was always shown to be possible solely if the HC is acquired first, the study of a circulative nanovirus (Di Mattia et al., 2022) proved that the reverse sequence also works in this viral system. At this stage, it is not clear whether this observation is inherent to a distinct mode of action of HCs in circulative viruses, because the only other HC characterized for another circulative virus, the phenuivirus RSV (Lu et al., 2019), has not been tested when acquired after the virus particles.

The sequential acquisition has been proposed as a key feature of HCs because it allows HC-trans-complementation, *i.e*. the HC encoded by a genome X in a virus population can assist the transmission of a genome Y from the same population or from another population in another host plant, allowing cooperation between related viral genomes at the transmission step rather than competition (Pirone & Blanc, 1996). It is noticeable that, in this view, the sequence of acquisition does not matter, and so the current definition of HCs still perfectly holds for all cases reported to date.

5.2 How does a helper component work

The sole mode of action of HCs reported to date is the bridge hypothesis extensively described in previous sections. This hypothesis derives from the observation that HCs have to be present for the virus particle to be efficiently retained or internalized in the vector. Without HC, virions are supposedly flushed together with the flux in the digestive track, explaining why the HC have to be acquired either prior to or together with the virus particles. Clearly, the fact that the HC of nanoviruses (the NSP protein) can efficiently mediate the aphid transmission of virus particles acquired several days ahead is questioning its mode of action. The virus particles could somehow be retained within the gut lumen of aphid vectors and be internalized and accumulate within epithelial gut cells solely through the action of the protein NSP. This is opening several possibilities that are calling for further investigation. The virus particles could be retained in the gut lumen non-specifically, embedded in gut cell secretions or stuck in between the microvilli, but they could also be specifically attached to membrane receptors. Investigating the viral determinants of nanovirus-aphid specificity would answer this question. Then the NSP protein acquired later could trigger internalization of virions or of NSP-virion complexes. Additional role of NSP in transcytosis, and release of virus particles into the hemolymph are also conceivable and would all represent a mode of action significantly distinct from the classical bridge hypothesis.

5.3 Why are helper components so frequently involved in the vector transmission of plant viruses

The distinction between capsid and helper strategies in vector-transmission of viruses has been conceptualized and empirically studied solely in plant virology (Blanc & Gutiérrez, 2015). The requirement of an HC for successful virus-vector interaction have been confirmed countless times, on totally unrelated viruses, transmitted by unrelated vectors, through different mechanisms (Hogenhout et al., 2008; Dietzgen et al., 2016; Lu et al., 2019). Therefore, the helper strategy certainly evolved several times independently, indicating that it likely confers a selective benefit, at least in some environmental conditions. Paradoxically, because it requires the production of an additional viral protein to ensure virus transmission, the helper strategy may incur a cost to the virus when compared to the capsid strategy. However, some HCs interact with various partners (other viral, host plant or vector molecules) at different steps of the virus cycle, either in the vector or within the host plant where they ensure important and diverse functions. A case study is the multi-tasking protein HC-Pro of potyviriruses involved in many different processes (Valli et al., 2018)**.** This multifunctionality may compensate for the cost associated to the maintenance of a helper protein and may instead confer specific benefit to viruses. But not all HCs are known to be multifunctional and so the benefit they may provide remains largely elusive. Their possible multifarious mode of action in distinct viral clades may stem from the fact that each of these clades have evolved HCs with their own molecular means, and the question of whether the selective pressure that drove this evolution are similar in all cases is an interesting issue. In other words, whether all HCs have been selected for the same reason, and for what reason, is a complete mystery for which some speculations are provided below.

Cooperation hypothesis

A long-standing proposition is that HCs could result from selection at the quasispecies/virus population level (Pirone & Blanc, 1996). According to these authors, HC allows cooperation between genomes of a viral population (or of distinct populations located in different host plants) during vector transmission,

whereas the capsid strategy does not. If a genome within a population produces a HC that is adapted to the dominant vector species in a given place and at a given time, it will assist the transmission of other genomes acquired through HC-trans-complementation that do not produce such adapted HC. The consequence is the relaxing of the bottleneck associated to selection by the vector and maintenance of a higher polymorphism in the transmitted population. This hypothetical benefit could apply to all virus species with the helper strategy, but unfortunately no experimental support has been provided, and a way to test for it is even hard to imagine.

Collective transmission hypothesis

An important proportion (that can be up to 100%) of virions from viral populations of most virus species appears to contain defective genomes (for reviews on defective particles see (Sun & Brooke, 2018; Vignuzzi & López, 2019), or only part of the genetic information (for a review on multipartite virus see (Michalakis & Blanc, 2020), resulting in a possible benefit of co-transmitting several particles to the same host (or cell within this host) in order to restore infectivity. This view of a viral infectious unit as a "collective infectious unit" has been extensively reviewed, and in particular the mechanisms through which viruses can achieve collective transmission (Sanjuán & Thoulouze, 2019; Shirogane et al., 2019). While the mechanisms invoked include the incorporation of several genomes in the same capsid (bacteriophage), the passage of large amounts of virus particles through virus-induced nanotubes connecting distinct cells or through membrane vesicles transferring in viral synapses (HIV, Influenza, Herpesvirus), the formation of virion aggregates with spontaneous binding between particles (vesicular stomatitis virus), or the multi-virion complexes found in occlusion bodies of baculoviruses, HCs have not been hypothesized to potentially facilitate collective transmission. Most characterized HC have been demonstrated to bind the virus coat protein (Blanc et al., 1997; Peng et al., 1998; Plisson et al., 2005; Hoh et al., 2010; Lu et al., 2019; Ji et al., 2019), to also self-interact (Hebrard et al., 2001; Ruiz-Ferrer et al., 2005; Hepojoki et al., 2010; Krenz et al., 2017), and thus to possibly trigger aggregation of virus particles. For example, we have demonstrated that the genome segments of nanoviruses acquired simultaneously (Di Mattia et al., 2020) or even sequentially (Di Mattia et al., 2022) by the aphid vector accumulate all together in the same intracellular aggregates in the AMG cells solely if a functional segment N encoding the NSP protein is present in infected plants. Nanoviruses being multipartite, collective transmission of several viral particles may appear immediately beneficial (Michalakis & Blanc, 2020). However, HCs have been described in numerous monopartite viruses, suggesting that, under this hypothesis, collective transmission would be beneficial whatever the genome architecture/organization.

The effector hypothesis

When entering a cell, viruses have to manipulate its defenses to initiate infection (Wu et al., 2019). This is true for many intracellular pathogens, including bacteria and fungi, which release effector proteins hampering cell-defense and facilitating infection. When injected into a new plant by a vector, one viral protein that is first in contact with the host cell is the coat protein. While its putative contribution to counter cell defenses has been envisaged and discussed (Conti et al., 2017; Nicaise & Candresse, 2017; Wu et al., 2019), that HCs could also be released together with virions and act as early pathogen effectors has not been envisaged. Most if not all HCs of non-circulative and circulative viruses could be multifunctional proteins. Beyond constituting a molecular bridge linking the virus particles to vector receptors, HCs also frequently have additional features that could reveal effector activity. Again, the emblematic example is the HC-Pro of potyviruses that can hinder the plant RNAi machinery, but other HCs, less understood, can also block the microtubular network (P2 of CaMV) (Blanc et al., 1996), induce membrane fusion (NSvc2C of RSV) (Lu et al., 2019) or interact with stress granules (NSP of PNYDV) (Krapp et al., 2017). That they interact with highly symmetrically arranged coat proteins, either icosahedral or helicoïdal, offers multiple attachment sites per particle and ensures that a high number of HCs can potentially be retained in and be released from the vector together with virions. The amount of HC molecules delivered could even be larger when HCs are prone to self-oligomerization as is the case for HC-Pro of potyviruses, P2 of CaMV, NSP of nanoviruses and NSvc2 of RSV. Hence, mobilizing a limited number of receptors in the vector may allow hundreds of HC molecules to be retained and/or internalized and later inoculated into the plant. The present paradigm regarding the primary role of an HC could completely change by considering that it could be an effector useful to the virus upon arrival in a cell and that its binding to the virus particles and to the

vector would merely ensure that it travels and is delivered with it. Whether HCs are released together with virions within host plant cells is not known but investigation of this question would be relevant for this hypothesis. It is also important to note that the “effector” hypothesis could apply to all reported HCs, and that the effector function may either act in host plant cells, in vector cells, or both.

6. Conclusion

In conclusion, several potential benefits associated to the helper strategy are imaginable. We insisted in this conclusion that each of this benefit could apply to all cases described, whatever the viral clade concerned. However, we cannot exclude that distinct helpers have evolved for distinct reasons and perhaps, other potential explanation are still to be conceived. Unfortunately, as they stand, the here-described hypothesis, with perhaps the exception of the “effector hypothesis” are not empirically testable, maintaining the question of the raison d’être of HCs a major conceptual challenge for future research in plant virology and vector transmission.

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Conflict of Interest disclosure

The authors of this preprint declare that they have no financial conflict of interest with the content of this article.

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