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Dicistrovirus–Host Molecular Interactions

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Abstract

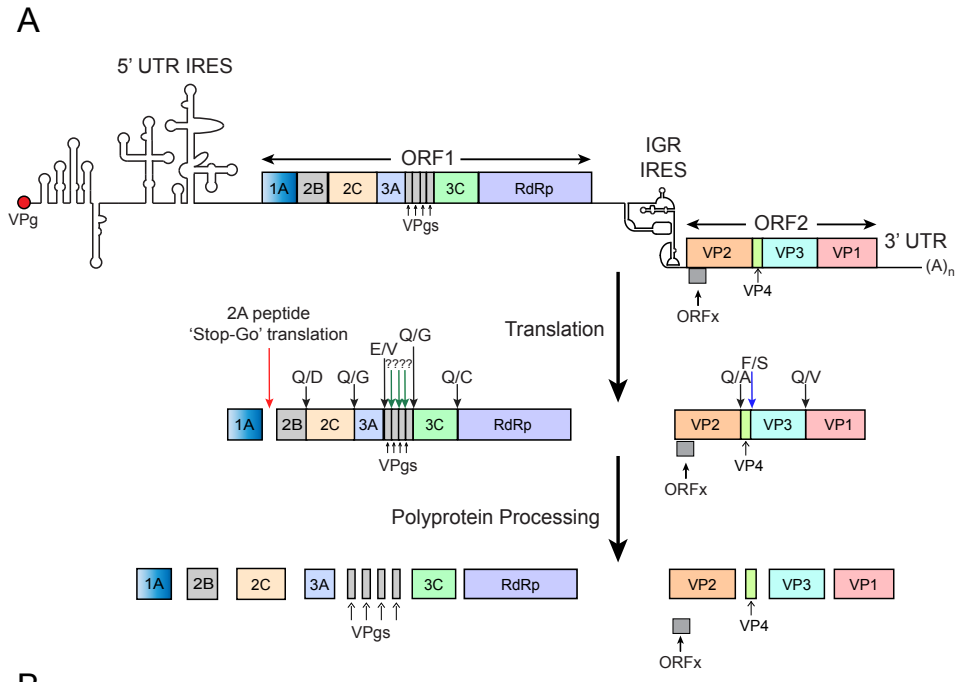
Members of the family *Dicistroviridae* are small RNA viruses containing a monopartite positive-sense RNA genome. Dicistroviruses mainly infect arthropods, causing diseases that impact agriculture and the economy. In this chapter, we provide an overview of current and past research on dicistroviruses including the viral life cycle, viral translational control mechanisms, virus structure, and the use of dicistrovirus infection in *Drosophila* as a model to identify insect antiviral responses. We then delve into how research on dicistrovirus mechanisms has yielded insights into ribosome dynamics, RNA structure/function and insect innate immunity signalling. Finally, we highlight the diseases caused by dicistroviruses, their impacts on agriculture including the shrimp and honey bee industries, and the potential use of dicistroviruses as biopesticides. Although knowledge of the mechanisms underlying dicistrovirus virus–host interactions is limited, the establishment of the first infectious clone should accelerate the discovery of new mechanistic insights into dicistrovirus infections and pathogenesis.

Introduction

Dicistroviruses are small plus-strand RNA viruses that primarily infect arthropods. They are classified into three genera: *Cripavirus*, *Aparavirus* and *Triatovirus*. Initially discovered in 1963 with the identification of *Acute bee paralysis virus* in honey bees (Bailey *et al.*, 1964), they were initially thought to be picornaviruses due to the similarly small size of the virion and genome, and disease symptoms (i.e. paralysis). However, it is now apparent that dicistroviruses belong to a unique virus family. Dicistroviruses have been identified in many orders of arthropods: Coleoptera (Reinganum, 1975), Lepidoptera (Reinganum, 1975), Orthoptera (Wilson *et al.*, 2000b), Diptera (Jousset *et al.*, 1972), Hemiptera (D'Arcy *et al.*, 1981a; Muscio *et al.*, 1987; Williamson *et al.*, 1988; Toriyama *et al.*, 1992; Nakashima *et al.*, 1998; Hunnicutt *et al.*, 2006), Hymenoptera (Bailey *et al.*, 1963; Bailey and Woods, 1977; Maori *et al.*, 2007; Valles and Hashimoto, 2009), Decapoda (Hasson *et al.*, 1995). Dicistroviruses provide for an excellent model system for studying fundamental virus–host interactions including viral translational control mechanisms and innate insect immune pathways. *Dicistroviridae* are of economic and agricultural importance; infections can lead to disease and outbreaks in arthropods, including in shrimp and honey bees. As the name implies, dicistroviruses contain a bicistronic monopartite RNA genome encoding two main open reading frames (ORFs). However, recent evidence indicates that some members contain a third ORF, termed ORFx. In this chapter, we provide an overview of dicistrovirus life-cycle, viral translational strategies, host interactions that modulate cellular pathways and innate immune responses, pathogenesis, and the potential use of these viruses as biopesticides.

Genome structure

Dicistroviruses have single-stranded, positive-sense RNA genomes of 8–10 kb in length and encoding two long ORFs (Fig. 5.1). The ORFs are translated as polyproteins that are then processed into the individual mature viral proteins by the virally-encoded protease 3C, a 2A 'stop-go' translation mechanism and other yet to be identified



B

ORFx sequences

CrPV: KMKIKDLCQNRKKLYILLAKELPLVLLRSLISLIFQLIIWT (41 amino acids (aa))

IAPV: AIHNKKAILPTYTIRNSLRPLVKTRLRPKKSQPFMMWKLQIGSIPPWLRILHRLGTWMIRTVLFSFY
SAPFSLTTLRSLLEQRPMQTNPLADMC (94 aa)

ABPV: PIKKQILPTYIIRNSRRPLKKTQLKRNKSPPFMMWKLQIGSIPPWLKTLHRLGAWMIRTVLFSFYNAP
YSLTTLRSLLDQQQMITNPSIDMC (92 aa)

KBV: LITKKTILPMYITRNSRRPQRRMPLRRNKSPPFMMWKLQIGSIPPWLRILHRLGAWMIRTVLFSFYN
APFSLTTLRLLQEQLPITTQHSVDMC (93 aa)

SINV-1: LNQTMRILLRRKRQQPLLPTHNRNLARRPQKIPLPDKNSQFSMMLNNLASLFQLLRKRLALLLSLIL
QRQLWIFFLELLSSINSSLFKVNQTITTNPLTQQLLKTPNQPSDSIPCQETFLSWVAS (125 aa)

Figure 5.1 (A) The RNA genome and polyprotein processing of dicistroviruses. The positive sense, single-stranded RNA genome is shown above. The RNA genome is translated by the 5′UTR and IGR IRESs to produce two large polyproteins that are then processed by proteases. Open reading frames are labelled as ORF1 and ORF2, encoding non-structural proteins (1A, 2B, 2C, 3A, 3B, 3C, RdRp (3D), VPgs and structural proteins (VP1-VP4). The CrPV 5′UTR and IGR IRES structures are shown (Gross *et al*., 2017). Dicistroviruses contain one to six VPgs. Shown is the CrPV genome containing 4 VPgs. A subset of dicistroviruses including IAPV, KBV, ABPV and SINV-1 contains a short +1 frame open reading frame called ORFx that overlaps with the 0 frame ORF2 adjacent to the IGR IRES. The IGR IRES directs both 0 and +1 frame translation. Polyprotein processing occurs co-translationally and post-translationally (red arrow). The 2A peptide, sequence located between 1A and 2B, directs 'stop go' translation separating 1A from polyprotein. Black arrows: Cleavages mediated by the virally encoded 3C protease at the indicated cleavage sites preferring cleavage after a Q residue. Blue arrow: Predicted cleavage site. Green arrows: Processing events that have not been characterized. (B) The ORFx amino acid sequences of CrPV, IAPV, ABPV, KBV and SINV-1 from accession numbers NC_003924.1 (CrPV), EF219380.1 (IAPV), AF150629 (ABPV), AY275710 (KBV), AF277675 (SINV-1).

mechanisms. The upstream ORF, termed ORF1, encodes non-structural proteins that modulate host cellular processes and evade innate immune responses, thereby facilitating virus infection. ORF1 encodes the viral silencing suppressor 1A protein, the 2C RNA helicase, the viral genome-linked protein VPg (King and Morre, 1988), the 3C protease, and the RNA-dependent RNA polymerase (RdRp) 3D (Bonning and Miller, 2010) (Table 5.1). ORF1 also encodes 2B and 3A proteins that, based on sequence homology to other viral proteins, are thought to be involved in the assembly of the viral replication complex membrane and in membrane remodelling. The roles of the 1A protein during infection are beginning to be understood (see below), whereas the functions of many of the other non-structural proteins have yet to be elucidated in detail. The downstream ORF2 encodes the structural proteins VP2, VP1, and VP3 that are involved in encapsidation of the virion, as well as VP4 (Tate *et al.*, 1999; Sanchez-Eugenia *et al.*, 2005). The RNA genome also contains a 5′ linked viral protein VPg and a 3′ poly A tail (King and Moore, 1988).

Translation of the ORFs is mediated by two distinct internal ribosome entry sites (IRES): a 5′ untranslated region (5′UTR) IRES and an intergenic (IGR) IRES, the latter of which has been studied extensively at the biochemical and structural levels. A subset of dicistroviruses, specifically the honey bee viruses, *Israeli acute paralysis virus* (IAPV), *Kashmir bee virus* (KBV) and *Acute bee paralysis virus* (ABPV), the fire ant virus, *Solenopsis invicta virus* (SINV-1) and *Cricket paralysis virus* (CrPV), encode an overlapping +1 frame ORF termed ORFx within ORF2, downstream of the IRES (Firth *et al.*, 2009; Sabath *et al.*, 2009; Ren *et al.*, 2014; Au *et al.*, 2015; Kerr *et al.*, 2018b) (Table 5.2). During dicistrovirus infection, the structural proteins are produced in supramolar excess over the non-structural proteins, suggesting that translation of ORF1 and ORF2 are differentially regulated by the IRESs (Moore *et al.*, 1980; Wilson *et al.*, 2000b; Khong *et al.*, 2016).

Dicistroviruses encode up to six copies of the genome linked protein VPg (King and Moore, 1988; Nakashima and Shibuya, 2006). Based on studies of other RNA viruses, VPgs have direct roles

Table 5.1 Functions of dicistrovirus proteins

Viral proteins	Known or predicted* functions	Reference
1A (CrPV, DCV)	Inhibition of RNA interference	Nayak *et al.* (2010)
1A (CrPV)	Inhibition of SG assembly	Khong *et al.* (2011, 2017)
2B	Membrane remodelling*	Aldabe *et al.* (1996)
2C	RNA helicase, membrane permeabilization*	Gorbalenya *et al.* (1990); Mirzayan *et al.* (1994); Rodriguez *et al.*(1993)
3A	Viral replication complex formation*	Fujita *et al.* (2007)
VPgs	Covalently linked to 5' end of genomic RNA for priming viral replication	King and Morre (1988)
3C (PSIV)	Viral poly protein processing	Nakashima *et al.* (2008)
3D	Negative strand RNA synthesis, viral replication	Bonning and Miller (2010)
ORFx (IAPV, ABPV, KBV, SINV-1)	?	Au *et al.* (2015); Firth *et al.* (2009); Ren *et al.* (2014); Sabath *et al.* (2009)
VP1-3	Capsid proteins, virion structure	Tate *et al.* (1999)
VP4 (TrV)	Membrane permeabilization during viral entry	Sanchez-Eugenia *et al.* (2005)
? (CrPV)	Host translation shut off	Garrey *et al.* (2010)
? (CrPV, DCV)	Induction of JAK-STAT pathway	Dostert *et al.* (2005)
N terminal region of ORF1(TrV)	Induction of apoptosis	Mari *et al.* (2002)
? (DCV)	Induction of heat shock responses	Merkling *et al.* (2015)

*Predicted functions based on sequence alignment.

Table 5.2 Members of *Dicistroviridae*

Genus[1]	Members[2]	Natural host[3]	IGR IRES type[4]	ORF2 start codon[5]	+1 Frame ORFx[6]	ORFx length[7]	Reference[8]
Aparavirus	*Acute bee paralysis virus** (ABPV)	Honey bee	II	GCC (Ala)	Yes (CCG, Pro)	92	Govan *et al.* (2000)
	Israeli acute paralysis virus (IAPV)	Honey bee	II	GGC (Gly)	Yes (GCG, Ala)	94	Maori *et al.* (2007)
	Kashmir bee virus (KBV)	Honey bee	II	GCC (Ala)	Yes (CUG, Leu)	93	de Miranda *et al.* (2004)
	Mud crab dicistrovirus (MCDV-1)	Mud crab	II	GCU (Ala)	? (CUC, Leu)	9	Guo *et al.* (2013)
	Solenopsis invicta virus 1 (SINV-1)	Fire ant	II	GCU (Ala)	Yes (CUC, Leu)	125	Valles *et al.* (2004)
	Taura syndrome virus (TSV)	Penaeid shrimp	II	GCU (Ala)	? (CUA, Leu)	16	Mari *et al.* (2002)
Cripavirus	*Aphid lethal paralysis virus* (ALPV)	Aphid	I	GCU (Ala)	? (CUA, Leu)	7	Van Munster *et al.* (2002)
	*Cricket paralysis virus** (CrPV)	Cricket+	I	GCU (Ala)	Yes (AAA, Lys)	41	Wilson (2000)
	Drosophila C virus (DCV)	Fruit fly	I	GCU (Ala)	? (CUA,Leu)	51	Johnson Christian (1998)
	Rhopalosiphum padi virus (RhPV)	Aphid	I	GCA (Ala)	? (CAA, Glu)	2	Moon *et al.* (1998)
Triatovirus	*Black queen cell virus* (BQCV)	Honey bee	I	GCU (Ala)	? (CUG, Leu)	26	Leat *et al.* (2000)
	Himetobi P virus (HiPV)	Planthopper	I	GCA (Ala)	? (CAA, Glu)	14	Nakashima, Toriyama (1999)
	Homalodisca coagulata virus 1 (HoCV-1)	Leafhopper	I	GCA (Ala)	? (CAA, Glu)	14	Hunnicutt (2006)
	Plautia stali intestine virus (PSIV)	Stink bug	I	CAA (Gln)	? (AAG, Lys)	43	Sasaki *et al.* (1998)
	*Triatoma virus** (TrV)	Triatomine bug	I	GCU (Ala)	? (CUG, Leu)	1	Czibener *et al.* (2000)

[1,2]Genus and members of *Dicistroviridae* based on ICTV classification.
[3]Natural hosts based on experimental and observational evidence.
[4]Type of intergenic internal ribosome entry site (IGR IRES).
[5]Start codon of ORF2 based on experimental evidence and/or phylogenetic analysis.
[6]Yes, experimentally validated; ?, has not been tested.
[7]Predicted ORFx amino acid length.
[8]Reference of first published sequence.
+CrPV has a wide range of hosts, including in the orders Diptera, Hemiptera, Hymenoptera, Lepidoptera, Orthoptera
*Indicates type species.

in replication, acting as a primer, and in viral translation (Goodfellow, 2011). Although it is unclear why there are redundant copies in some dicistroviruses, studies on other multi-VPg-containing RNA viruses, such as the picornavirus, *Foot-and-mouth disease virus*, suggest that the multiple VPgs ensure that each progeny RNA molecule is linked to a VPg for efficient replication (Falk *et al.*, 1992). Because translation of non-structural proteins from ORF1 is much lower than that of ORF2, it may be that multiple copies are needed to compensate for the lower expression of non-structural proteins during dicistrovirus infection (Nakashima and Shibuya, 2006).

Taxonomy

Though originally proposed to be picorna-like viruses, it is now apparent that dicistroviruses

belong to a distinct viral family based on their bicistronic genome arrangement as compared to the picornavirus monocistronic genome. Moreover, dicistrovirus non-structural proteins are upstream of the structural proteins, the reverse of picornaviruses. *Dicistroviridae* is one of six families belonging to the order *Picornavirales* of (+) ssRNA viruses. Based on alignments of the RdRp protein sequences, they are most similar to members of the subfamily *Comoviridae*, plant viruses containing two genomic RNAs: one encoding the structural proteins and the other encoding the non-structural proteins (Chen and Siede, 2007; Koonin *et al.*, 2008). At the time of writing, there are three genera within the *Dicistroviridae* based on phylogenetic analysis of the IGR IRES as classified by the International Committee on Taxonomy of Viruses (ICTV): *Cripavirus*, *Aparavirus* and *Triatovirus* (Valles *et al.*, 2017) (Table 5.2 and Fig. 5.2). *Aparavirus* is further divided into distinct, divergent clades: the insect-infecting aparaviruses and the crustacean-infecting aparaviruses. As more dicistrovirus sequences are classified (Shi *et al.*, 2016) *Aparavirus* may be reclassified into two distinct genera based on differences in host, details of the IGR IRES and the presence of an ORFx (Firth *et al.*, 2009; Sabath *et al.*, 2009).

Members of the genus *Aparavirus* include the honey bee infecting IAPV, KBV and ABPV, the red fire ant infecting SINV-1 and the penaeid shrimp-infecting *Taura syndrome virus* (TSV). Several of these aparaviruses have had a negative impact in agriculture and on the economy (see below). Dicistroviruses also have indirectly linked to human health. For example, SINV-1 has been proposed as a possible biopesticide for fire ant infestations (Valles, 2012), and the *Triatoma virus* (TrV), a member of the genus *Triatovirus*, can infect Triatominae, which can transmit *Trypanosoma cruzi*, the parasite that causes Chagas disease (Marti *et al.*, 2017). Members of the genus *Cripavirus*, including *Cricket paralysis virus* (CrPV) and *Drosophila C virus* (DCV), have wide host ranges, including the genetic model organism *Drosophila melanogaster* (Plus *et al.*, 1978; Masoumi *et al.*, 2003). As such, CrPV and DCV infections of *D. melanogaster* are

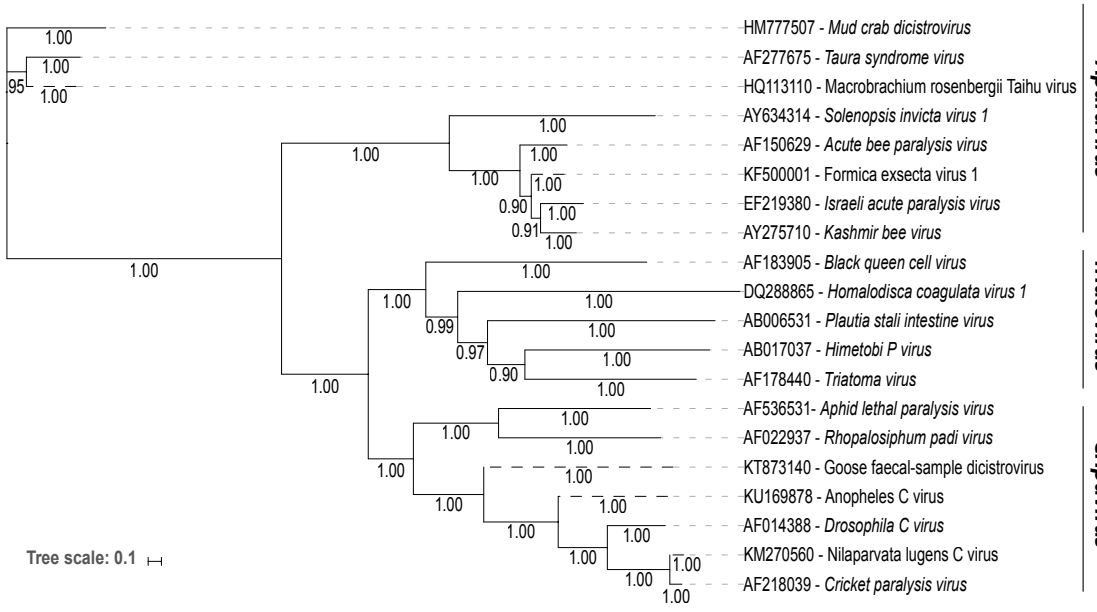


Figure 5.2 Phylogenetic tree of dicistroviruses classified by the ICTV. Midpoint rooted phylogenetic tree constructed from full-length structural polyprotein amino acid sequences of accepted and classified dicistroviruses. In addition, a few unclassified metagenome-derived sequences were included and are rooted using dashed lines. Analysis was performed using Bayesian inference of phylogeny. Bootstrap probability values to two decimal places are given and labelled at the nodes. The current genera, as described by ICTV are shown. Analysis was run by ICTV (Valles *et al.*, 2017) and the tree was manipulated using iTOL (Letunic and Bork, 2016).

used successfully as models for studying fundamental dicistrovirus–host interactions.

In a landmark transcriptomics paper, several hundred invertebrate viruses were discovered from a wide variety of invertebrate species based on sequence alignment using RdRps, including over a hundred novel dicistro-like viruses (Shi *et al.*, 2016). Although the new dicistro-like viruses remain to be classified by ICTV, the identification of these new genomes highlights the diversity of this viral family across a wide spectrum of host species.

Virion structure

The structures of the dicistroviruses CrPV (Tate *et al.*, 1999), TrV (Squires *et al.*, 2013), IAPV (Mullapudi *et al.*, 2016), and BQCV (Spurny *et al.*, 2017) have been solved (Fig. 5.3). Dicistroviruses are non-enveloped viruses that are roughly spherical, approximately 30 nm in diameter and exhibit icosahedral, $T = 3$ symmetry comprising of 60 protomers, each consisting of the three major capsid proteins, VP1, VP2 and VP3. The virions are structurally similar but not identical. Each capsid protein contains a jelly roll structure comprised of eight β sheets in a β barrel arrangement. VP1 subunits form a pentamer around a 5-axes fold, and the VP2 and VP3 form heterohexamers around the icosahedral 3-fold axes. The structures also revealed that the small protein VP4 is attached to the inner surface of the viral asymmetric unit and is located between the protein shell and RNA within the capsid, possibly contacting the viral genome. VP4 is also present in crystallized virions that are infectious.

Dicistroviruses assemble as immature particles containing the precursor protein VP0, which consists of VP3 and VP4. Cleavage of VP0 into VP3 and VP4 is proposed to occur after the particles encapsidate the viral RNA genome, supported by the observation that empty capsids contain unprocessed VP0 (Tate *et al.*, 1999). A conserved Asp-Asp-Phe (DDF) motif within VP1 protein may be involved in this cleavage process (Squires *et al.*, 2013; Mullapudi *et al.*, 2016; Spurny *et al.*, 2017). The DDF motif is in close proximity of the N terminus of VP3 and the C terminus of VP4, suggesting that this motif catalyses this cleavage. Interestingly, TrV VP3 contains a second autoproteolytic DDF motif in addition to the conserved motif within VP1 (Squires *et al.*, 2013). The function of this additional motif has yet to be elucidated.

Dicistrovirus virions are stable in acidic conditions (to pH 3.0) whereas alkaline conditions (pH > 8.0) trigger uncoating by releasing the interactions

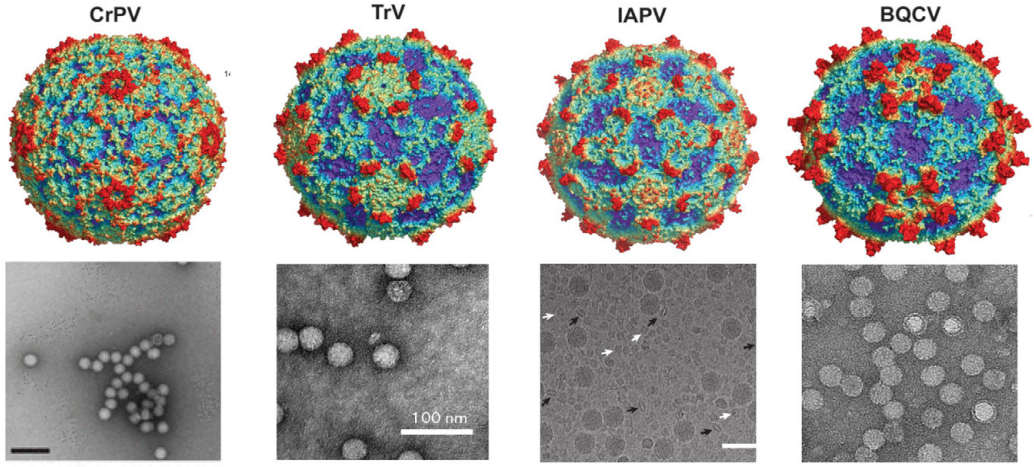


Figure 5.3 Dicistrovirus structures. Shown are crystal structures of capsids from CrPV, TrV, IAPV and BQCV (Spurny *et al*., 2017). PDB structure ID: 1B35, 3NAP, 5LWG and 5MQC. Negative stained electron micrographs of purified CrPV (Kerr *et al*., 2015), TrV (Valles *et al*., 2017), IAPV (Mullapudi *et al*., 2016) and BQCV (Spurny *et al*., 2017). Figures are republished with permission from Kerr *et al*. (2015; Copyright © 2015, American Society for Microbiology. All Rights Reserved. doi:10.1128/JVI.00463–15), Mullapudi *et al*. (2016), Spurny *et al*. (2017) and Valles *et al*. (2017).

between capsid proteins, as demonstrated using TrV virions (Snijder *et al.*, 2013). Uncoating occurs through an intermediate step in which the virions disassemble into pentons, with concomitant release of VP4.

Transmission

While the majority of dicistroviruses are transmitted horizontally via a faecal–oral route (Jousset *et al.*, 1972; Gomariz-Zilber *et al.*, 1995; Muscio *et al.*, 2000; Chen and Siede, 2007), some dicistroviruses may be transmitted vertically. For example, CrPV can be transmitted vertically transovum (with the virus on the surface or inside of the egg) (Reinganum *et al.*, 1970). ALPV, *Plautia stali intestine virus* (PSIV), TrV and SINV-1 can all be transmitted vertically by transovarial transmission, i.e. within the ovary with the virus inside the egg (D'Arcy *et al.*, 1981b; Hatfill *et al.*, 1990; Muscio *et al.*, 1997; Nakashima *et al.*, 1998). Viruses of sap-sucking insects (Hemiptera) such as RhPV, can be transmitted via plants. RhPV uses plants as a vehicle for spread, by circulating in the phloem of the host plant from which it can be acquired by other aphids (Gildow and d'Arcy, 1988; Ban *et al.*, 2007). The dicistro-like Big-Sioux River virus (BSRV) has also been found in maize (Ban *et al.*, 2007; Wamonje *et al.*, 2017). These examples highlight the use of plants as vectors for the spread of dicistroviruses to sap-sucking insects.

Viral entry, polyprotein processing and replication

Viral entry

Molecular details of dicistrovirus entry and replication have mostly come from studies of DCV. Using DCV-infected *Drosophila* S2 cells as a model, viral entry occurs through the clathrin-mediated endocytosis pathway and involves nuclear protein Bub1 (Budding uninhibited by benzimidazoles 1), a highly conserved subunit of the kinetochore complex that regulates chromosome congression (Cherry and Perrimon, 2004; Yang *et al.*, 2018). DCV infection results in more Bub1 translocated to the membrane, supporting a model where Bub1 regulates endocytosis to promote viral entry, possibly interacting with a clathrin-dependent endocytic compartment. In support of this, *bub1* deficient flies are resistant to DCV infection (Yang *et al.*, 2018). Cell attachment and entry is likely mediated through an initial attachment with a host cell surface receptor, which has yet to be identified. Following entry, the dicistrovirus uncoats, releasing its genome into the cytoplasm; details of this process are beginning to emerge. Purified VP4 capsid protein from TrV can induce permeabilization by inserting into the membrane and forming discrete pores, which may enable cell entry or genome transfer during virus infection (Sánchez-Eugenia *et al.*, 2015). Cryo-EM analysis of empty and full TrV particles has revealed that RNA release leads to empty capsids of similar size to the native virions that displayed no prominent conformational changes (Agirre *et al.*, 2013). According to the tectonic model for subunit movement, individual capsid proteins rotate within the capsid complex, leading to the disassembly of empty TrV capsids into small symmetrical, lip-shaped particles that are probably dimers of pentamers of capsid protein promoters. Cryo-EM structures of empty IAPV virions and virions that are about to release the viral genome suggest a distinct mechanism of genome release (Mullapudi *et al.*, 2016). The minor capsid proteins also detach from the capsid protein upon genome release. Finally, although structurally similar to the picornavirus capsid proteins, the CrPV capsid lacks the canyon that surrounds the five-fold symmetry identified in the poliovirus structure, which is important for poliovirus entry, suggesting a distinct mechanism of entry for CrPV (Tate *et al.*, 1999).

Polyprotein processing

Upon entry, the positive-strand dicistrovirus RNA genome is released and directly used as a template for viral protein synthesis (see below for detail on viral IRES translation). Indeed, transfection of an *in vitro* transcribed CrPV infectious RNA in *Drosophila* S2 cells can directly lead to viral protein synthesis and productive infection (Kerr *et al.*, 2015). Translation of the two main ORFs results in expression of polyproteins that are then processed into mature viral proteins (Moore *et al.*, 1980). The majority of the processing is mediated by the virally encoded 3C-like protease, a cysteine protease predicted to adopt a similar fold to picornavirus 3C protease (Nakashima and Nakamura, 2008). The 3C cleavage sites in the polyprotein of PSIV have been identified (Nakashima and Ishibashi, 2008,

2010). N-terminal sequencing of processed viral proteins showed cleavage occurs at Q/GWSLW and Q/NGVFD, which correspond to the 2A/3C and 3C/3D cleavage sites in the PSIV ORF1, respectively. The 2B/2C cleavage site has been mapped to amino acids 408–409 (Q/D), which are conserved among dicistroviruses. Q/A has also been observed at the putative cleavage junction of VP2/VP0. Collectively, these results indicate that the dicistrovirus 3C protease preferentially cleaves between Q/A, Q/G, Q/N and Q/D residues. In addition, the conserved Q/C, Q/A and Q/V sites between 3C/RdRp, VP2/VPO and VP0/VP1, respectively, are predicted to be cleaved by the 3C protease. However, this has not been experimentally validated (Fig. 5.1). Importantly, polyprotein processing can be recapitulated by incubating a CrPV infectious clone in an insect translation extract, thus providing a model to study protease-mediated cleavages (Kerr *et al.*, 2015). In a CrPV clone with a mutation that prevents 3C protease expression, ORF2 structural polyprotein processing is blocked, suggesting that ORF2 polyprotein is processed by 3C protease. However, ORF2 polyprotein could also be processed by other means. VP0 may undergo an autocleavage mechanism upon capsid formation involving a DDF motif present in dicistrovirus VP1 and in both VP1 and VP3 in TrV (Tate *et al.*, 1999; Agirre *et al.*, 2013).

Although the ability of 3C protease or a cellular protease to mediate cleavages at other junctions of the viral polyprotein is unclear, a subset of dicistroviruses including CrPV, DCV, IAPV, KBV and ABPV possess a 2A peptide sequence that mediates a 'self-cleavage' process. The 2A peptide sequence, located between 1A and 2B of ORF1, undergoes an unusual 'stop-go' translation mechanism leading to 'cleavage' of the protein (Luke *et al.*, 2008) (Fig. 5.1). The 2A peptide encodes a conserved DxExNPGP sequence, which interacts with the ribosome exit channel to induce stop-codon independent translation termination ending at the second to last glycine residue (Brown and Ryan, 2010). However, instead of the ribosome dissociating, the ribosome continues translation starting with a proline codon. The 2A peptide mechanism, which has been termed as 'stop-go' or 'stop-carry' translational recoding mechanism, is present in several positive-strand RNA viral genomes as a viral strategy to produce two protein products from a single polyprotein ORF. The 2A peptide of CrPV, DCV, IAPV, KBV and ABPV 'stop-go' translation has been experimentally tested *in vitro*, showing 88–95% cleavage activity (Donnelly *et al.*, 2001; Luke *et al.*, 2008). The conserved 2A peptide DxExNPGP sequence is present near the N-terminus of ORF1 in only a subset of dicistroviruses. It is not clear how those dicistroviruses lacking the 2A peptide mediate cleavage between the 1A and 2B non-structural proteins, nor why only a subset of dicistroviruses utilize this mechanism. Another remaining question is why the 2A peptide is utilized to separate 1A and 2B proteins, rather than a protease cleavage site.

Replication

Replication of the dicistrovirus RNA genome is mediated by the RdRp (3D) protein encoded within ORF1. A CrPV infectious clone containing a mutant catalytically-inactive RdRp does not support replication (Khong *et al.*, 2016). Like other RNA viruses, the plus strand RNA genome is used as a template for both translation and replication. RdRp synthesizes the negative strand RNA, which in turn transcribes the positive-strand RNA. DCV has been used as a model for study of dicistrovirus replication. Like other RNA viruses, DCV replication occurs on membrane structures, which are formed in part from remodelling of the Golgi apparatus to form a novel intracellular vesicular compartment where virus replication takes place (Cherry *et al.*, 2006). In support of this model, the coat protein complex 1 and fatty acid biosynthesis pathway are required for DCV replication. The vesicles are non-uniform in size, averaging 115 nm in diameter (Cherry *et al.*, 2006). Co-localization of the DCV helicase protein with the Golgi marker DG13 suggests that this is the site of viral replication (Cherry *et al.*, 2006). The molecular details underlying dicistrovirus replication have yet to be determined, but it is likely similar to picornaviruses, whose replication machinery involves RdRp in complex with or requiring host proteins and the viral-linked protein VPg, all of which interact with structural elements at the 5′ and 3′ ends of the dicistrovirus RNA genome (Paul and Wimmer, 2015). A recent study uses structural RNA probing approaches to begin modelling secondary structures at the 5′ end of the CrPV genome (Gross *et al.*, 2017). The importance of these secondary structures for replication has not been confirmed.

Packaging and egress

Encapsidation of the dicistrovirus RNA genome with capsid proteins has not been studied in detail; however, virion structures have been solved, which provide hints about this process (see section on virion structure). The release of the virus from infected cells also remains to be studied. Specifically, the mechanisms that underlie lytic versus non-lytic dicistroviruses are poorly understood, but the distinct dicistrovirus pathogenesis and transmission strategies probably dictate the route of viral egress.

Dicistroviruses are classified as nonenveloped viruses. An emerging theme is that classically defined nonenveloped viruses, such as Hepatitis A and C viruses and poliovirus, can hijack exosome-like or autophagic vesicles for virion release (Feng *et al.*, 2013; Ramakrishnaiah *et al.*, 2013; Bird *et al.*, 2014). A recent study shows that CrPV may also hijack the exosome pathway for viral release (Kerr *et al.*, 2018a). CrPV structural proteins and RNA are found in exosome-like vesicles (ELVs) and the ELVs from CrPV-infected cells are infectious. This suggests that the classically-defined nonenveloped viruses of invertebrates and vertebrates may acquire an envelope as a fundamental viral strategy to evade host immune responses and/or facilitate transmission. In this context, it will be interesting to determine whether the use of ELVs extends to other dicistroviruses.

Unique translation mechanisms for dicistroviral protein synthesis

All viruses must use the host ribosome for viral protein synthesis. Some viruses have evolved elegant strategies to hijack the ribosome (Jan *et al.*, 2016). One distinguishing feature of *Dicistroviridae* is their use of distinct IRESs to drive translation of the two ORFs (Wilson *et al.*, 2000b). Translation of the upstream ORF1 is mediated by the 5′UTR IRES, whereas downstream ORF2 translation is mediated by the intergenic IRES (IGR IRES). The IRES arrangement allows for differential regulation of expression of the non-structural and structural proteins during infection, presumably as a viral strategy to optimally coordinate the different steps of the viral life cycle (Wilson *et al.*, 2000b; Khong *et al.*, 2016). While the mechanisms underlying the 5′UTR IRES are emerging, the IGR IRES mechanism has been extensively studied at the biochemical and structural level. This has yielded tremendous insights into both ribosome function and the translational control strategies viruses use to hijack the host ribosome. We provide a brief overview of canonical cap-dependent translation before delving into dicistrovirus IGR and 5′UTR IRES-mediated translation.

Canonical translation versus internal ribosome entry sites

The majority of eukaryotic mRNAs utilize a cap-dependent scanning mechanism to recruit the ribosome and initiate translation from an AUG codon. Typically, an mRNA contains a 5′ m7G cap and a 3′ poly A tail, which act synergistically to mediate translational initiation and to protect the mRNA from ribonucleases (Jackson *et al.*, 2010; Hinnebusch *et al.*, 2016). There are ≈ 12 core translation initiation factors that orchestrate a sequential process of 80S ribosome assembly at the AUG start codon. Briefly, the 5′ cap is bound to the complex eukaryotic initiation factor 4F complex, consisting of the cap-binding protein, eIF4E, the RNA helicase eIF4A, and the scaffold protein eIF4G. eIF4G interacts with several proteins, including the eIF3/40S subunit and the poly A binding protein, the latter of which promotes translation by mediating circularization of the 5′ and 3′ ends of the mRNA. The ternary complex eIF2:Met-tRNA$_i$:GTP along with eIF1 and eIF1A are recruited to the 43S preinitiation complex (PIC) at the 5′ end. Scanning of the PIC occurs until the appropriate AUG codon is recognized via anticodon-codon base pairing of the Met-tRNA$_i$. The GTPase activating protein eIF5 is recruited to stimulate GTP hydrolysis on eIF2, thereby leading to dissociation of factors and 60S subunit joining to assemble an elongation-competent 80S ribosome. At this step, the AUG codon and Met-tRNAi occupy the ribosomal P site, leaving the A site empty to allow delivery of the first aminoacyl tRNA by elongation factor 1A. This begins the elongation step of translation.

An alternate mechanism of translation initiation is through an IRES. IRESs, found in a subset of viral and cellular mRNAs, can direct translation under virus infection and cellular stress when cap-dependent translation is compromised (Leppek *et al.*, 2018; Mailliot and Martin, 2018). IRESs are structured RNAs that use limited initiation

factors to recruit the ribosome. The limited factor requirement may allow IRES-containing mRNAs to be preferentially translated when global cap-dependent translation is inhibited. Viral IRESs have been studied extensively through reconstitution experiments and RNA structural probing analysis, revealing key conserved structural elements important for recruitment of the factors/ribosome. Viral IRESs are classified into four main groups based on these properties. The *Dicistroviridae* IGR IRES represents the most streamlined class of IRESs that can recruit the ribosome through an unprecedented mechanism.

Dicistrovirus IGR IRES – factorless IRES translation

The first hint that dicistrovirus IGR IRES is unique was the discovery that translation starts at a non-AUG codon (Sasaki and Nakashima, 1999; Wilson *et al.*, 2000a). For example, the CrPV IGR IRES initiates translation at a GCU alanine codon (Table 5.2 and Fig. 5.4). PSIV and CrPV IRESs have been modelled using extensive secondary structure modelling, RNA structural probing analysis and mutational analysis (Wilson *et al.*, 2000a; Kanamori and Nakashima, 2001; Jan and Sarnow, 2002). The core IGR IRES is approximately 180 nucleotides in length and adopts a triple pseudoknot (PK) RNA structure (Fig. 5.4). The RNA structure is separated into two domains that dictate function. PKII and PKIII domains are responsible for ribosomal recruitment, and PKI is responsible for ribosome positioning at the non-AUG start codon (Wilson *et al.*, 2000a; Kanamori and Nakashima, 2001; Jan and Sarnow, 2002; Costantino and Kieft, 2005; Kamoshita *et al.*, 2009) (Table 5.2). The IRES also contains unpaired nucleotides within loops and bulge regions (ex. L1.1, SLIV and SLV) that function to interact with specific regions of the ribosome (Fig. 5.4) (Pfingsten *et al.*, 2006; Schüler *et al.*, 2006; Jang *et al.*, 2009). Another unique aspect of dicistrovirus IGR IRES is that the IRES can directly

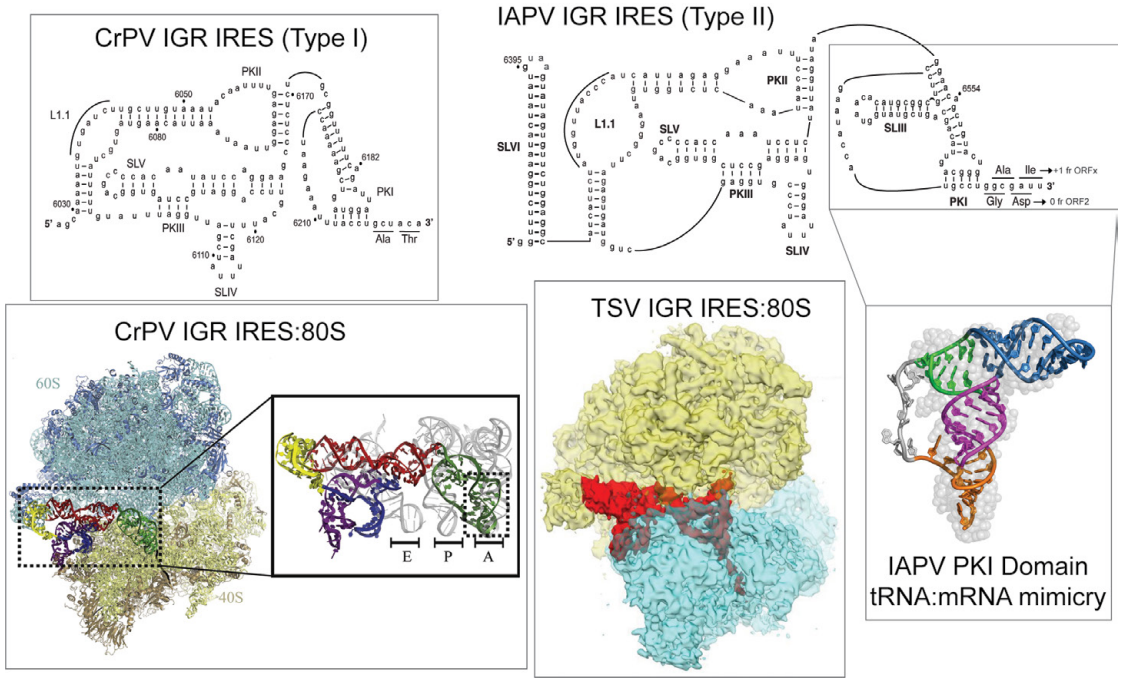


Figure 5.4 The dicistrovirus IGR IRES. (Top) The secondary structures of CrPV IGR IRES (Type I) and IAPV IGR IRES (Type II) are shown. (Bottom left) Cryo-EM structure of the Type I CrPV IGR IRES bound to the 80S ribosome (PDB ID 4V91). Figures republished from Fernandez *et al*. (2014), licensed under CC BY 3.0. (Bottom centre) Cryo EM structure of the Type 2 TSV IGR IRES bound to the 80S ribosome (PDB ID: 3J6Y). Figure republished with permission from Koh *et al*. (2014). (Bottom right) Global shape mimicry of tRNA by the IAPV IGR IRES PKI domain. The IAPV IGR IRES NMR/SAXS structure of the IAPV IRES PKI domain (PDB ID: 2N8V) is superimposed over $tRNA^{phe}$ (PDB ID:4TNA, in grey).

recruit the ribosome without initiation factors or initiator Met-tRNA$_i$ (Sasaki and Nakashima, 2000; Wilson *et al.*, 2000a). *In vitro* binding studies using purified ribosomal subunits show that the IRES binds to the purified 40S and 80S ribosomes tightly, with an affinity of 5–20 nM (Jan and Sarnow, 2002; Jan *et al.*, 2003). Reconstitution experiments using purified ribosomes, elongation factors, and aminoacyl-tRNAs showed that the IGR IRES can support translation in a minimal eukaryotic translation system (Jan *et al.*, 2003; Pestova and Hellen, 2003). Thus, the IGR IRES directly interacts with and manipulates the ribosomes to drive translation in *cis*, acting as its own mRNA template for protein synthesis.

The IGR IRESs are divided into Type I and II IRESs based on distinct elements. Type II IRESs contain a larger L1.1 domain, an extra stem–loop called SLIII within the PKI domain and lack a conserved UAC within the ribosome binding domain (Table 5.2, Fig. 5.4). Despite these differences, IRESs are functionally and structurally similar (Hatakeyama *et al.*, 2004; Cevallos and Sarnow, 2005; Pfingsten *et al.*, 2007; Nakashima and Uchiumi, 2009). Indeed, PKI domains can be functionally swapped between Type I and II IRESs (Jang and Jan, 2010; Hertz and Thompson, 2011).

Crystallography and cryo-EM structures of the IGR IRES have provided insights into the mechanism whereby the viral RNA actively manipulates the host ribosome to begin translation. The IGR IRES directly interacts with both the 40S and 60S subunits for 80S assembly, spanning all three tRNA ribosomal sites within the inter-subunit space of the 80S ribosome; PKII and PKIII occupy the E and P sites, while PKI occupies the A site (Spahn *et al.*, 2004; Schüler *et al.*, 2006; Fernández *et al.*, 2014; Koh *et al.*, 2014; Au *et al.*, 2015). Remarkably, the PKI domain, which positions the ribosome to start translation at a non-AUG codon, mimics a tRNA anticodon–codon interaction, thus explaining how the IRES occupies the ribosomal tRNA sites for initiation (Fig. 5.4) (Costantino *et al.*, 2008). Indeed, the PKI domain can be functionally swapped with a tRNA (Au and Jan, 2012). The PKII and PKIII domains adopt the compact globular core required for 40S and 60S recruitment to assemble the 80S ribosome (Pfingsten *et al.*, 2006; Schüler *et al.*, 2006). Specifically, SLIV and SLV contact ribosomal proteins rpS5 and eS25 of the 40S subunit, and the L1.1 domain interacts with the L1 stalk of the 60S subunit. Mutations within these domains block 40S or 60S subunit recruitment (Jan and Sarnow, 2002; Costantino and Kieft, 2005; Jang *et al.*, 2009).

The IRES also induces conformations within the ribosome that are important for IRES translation. For example, the ribosome bound to the IRES oscillates between canonical and rotated states, conformations that occur during translation normally (Fig. 5.5) (Fernández *et al.*, 2014; Petrov *et al.*, 2016). The rotated state involves a counterclockwise rotation of the 40S subunit relative to the 60S subunit. This state involves the 40S domain swivelling, as well as the movement of the L1 stalk of the ribosome. Since the L1.1 loop of the IRES interacts with the L1 stalk, it couples the IRES to the movements of the ribosome and forces it to adopt conformations that depend on the rotational state of the ribosome.

Cryo-EM reconstructions of the IRES:ribosome complexes undergoing translocation by addition of elongation factors and aminoacyl-tRNAs have revealed additional conformations that facilitate IRES translation (Muhs *et al.*, 2015; Abeyrathne *et al.*, 2016; Murray *et al.*, 2016). When the IRES is in a rotated state, elongation factor eEF2-GTP can bind to the ribosome, interacting with the ribosomal A site and PKI of the IRES, thus promoting translocation of the PKI domain from the A to P sites (Fig. 5.5). Delivery of the next aminoacyl-tRNA by the action of eEF1A stabilizes the IRES:ribosome such that the PKI is committed to the P site. Interestingly, without delivery of the next aminoacyl-tRNA, the IRES will undergo back-translocation whereby the PKI domain will translocate back to the A site. This entire process mediated by the IRES, called pseudotranslocation, is unique because it occurs without peptide bond formation. A recent cryo-EM study has reconstituted translocation of the IRESs to the ribosomal E site, thus IRESs undergo two translocation steps on the ribosome. This revealed a surprising conformational change within the IRES: the PKI domain flips from the decoding E site in the 40S to the 60S subunit, resembling an acceptor tRNA (Pisareva *et al.*, 2018). This remarkable finding suggests that the viral IRES undergoes dynamic RNA gymnastics to manipulate the ribosome. Importantly, all of these structural studies complement and support the extensive functional, mutational and biochemical

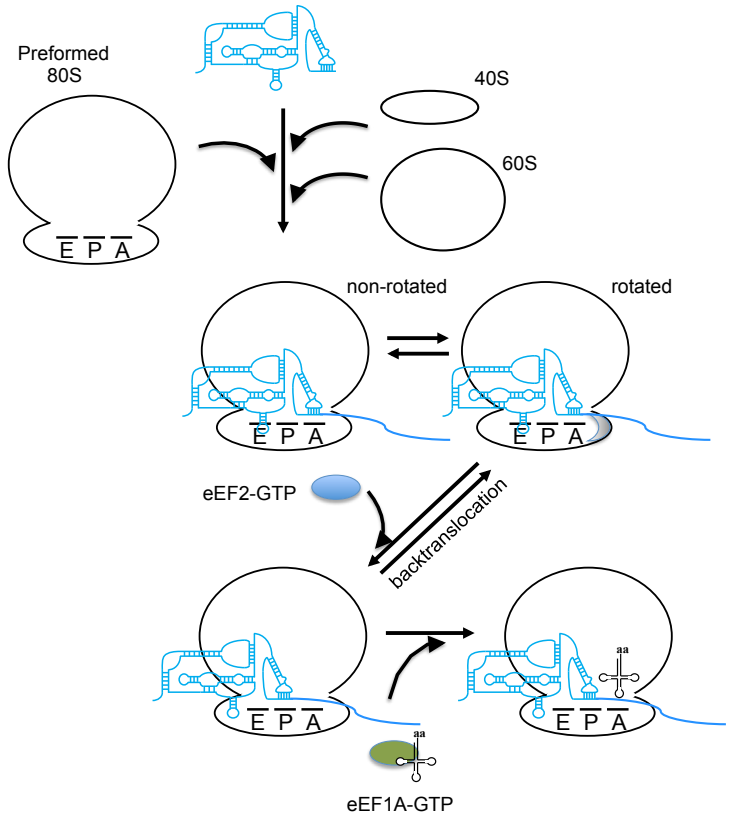


Figure 5.5 Model of IGR IRES mechanism. The 80S assembles on the IGR IRES through sequential recruitment of 40S and 60S or direct recruitment of a preformed 80S ribosome. Once in complex with the 80S ribosome, the ribosome alternates between a rotated and non-rotated state with the PKI tRNA anticodon:codon domain occupying the ribosomal A site. Pseudotranslocation occurs via the action of eEF2 with the PKI domain translocating from the P to A site of the ribosome. Back translocation of the PKI domain can occur unless there is delivery of the first aminoacyl-tRNA by eEF1A to lock the PKI domain in the ribosomal P site.

studies on the IRES (Yamamoto *et al.*, 2007). For example, mutations within the variable loop region (VLR) prevent pseudotranslocation (Ruehle *et al.*, 2015). In essence, the IRES hijacks the ribosome by 'tricking' the ribosome to start translation in the elongation mode of translation.

The use of single molecule fluorescence spectroscopy to capture the dynamics of IRES:ribosome interactions and conformational changes in real time have revealed that the IGR IRES can recruit 80S ribosomes through either sequential 40S then 60S binding or by recruitment of pre-formed 80S ribosomes (Petrov *et al.*, 2016) (Fig. 5.5). Monitoring the kinetic pathway of the initial steps of IGR IRES translation has revealed relatively slow initial translocation steps, which may suggest a quality control mechanism to ensure reading frame selection (Zhang *et al.*, 2016; Bugaud *et al.*, 2017). The future use of advanced biophysical approaches on IRES–ribosome complexes will provide new insights into this viral translational mechanism.

Although there have been studies on IGR IRES translation using reporter constructs in insect cell culture (Fernandez *et al.*, 2002; Wang *et al.*, 2013), the majority of studies of IGR IRESs have used *in vitro* approaches without the context provided by an *in vivo* virus infection. The development of the CrPV infectious clone allows reverse genetics approaches to test these *in vitro* models in a more physiologically realistic setting (Kerr *et al.*, 2015). Using this clone, mutations within the IGR IRES were tested for their effects on viral titre, translation and replication (Kerr *et al.*, 2016). Although most mutations tested that inhibited IRES translation *in vitro* correlated with a decrease in virus yield, some mutations did not correlate or could not be

simply explained by a defect in IRES translation. This study highlights the importance of *in vivo* studies to monitor IRES translation mechanisms in the context of viral infection.

In summary, IGR IRESs directly interact with and manipulate the ribosome by inducing coordinated conformational changes on the ribosome that resemble tRNA–ribosome interactions during translation in general. As such, studies on this factorless translation initiation mechanism will continue to shed light into fundamental ribosome functions and viral translational control strategies.

A third overlapping ORF – IGR IRES-mediated translation of alternative reading frames

Phylogenetic analysis revealed an extra overlapping +1 frame ORF (termed ORFx) within ORF2 of a subset of dicistroviruses which includes the honey bee viruses IAPV, KBV and ABPV as well as the fire ant virus SINV-1 (Firth *et al.*, 2009; Sabath *et al.*, 2009) (Table 5.2 and Fig. 5.1B). Subsequent investigations demonstrated that these IGR IRESs can direct translation in two reading frames: the 0 frame structural protein ORF2 and the +1 frame ORFx (Ren *et al.*, 2012). Using an *in vitro* translation system, the IAPV IGR IRES directed +1 frame translation at ~20% frequency of 0 frame translation. Specific elements within the PKI domain and an adjacent U:G wobble base pair mediate reading frame selection. RNA structural probing analyses suggest that the IRES also adopts distinct conformations to direct reading frame selection (Ren *et al.*, 2014; Au *et al.*, 2015). Finally, NMR/SAXS has revealed that the PKI domain of IAPV IRES mimics an RNA structure that resembles a tRNA shape, with SLIII acting as an acceptor stem of a tRNA (Fig. 5.4) (Au *et al.*, 2015). Thus, IGR IRESs not only hijack the ribosome for viral protein synthesis but can also direct translation in two reading frames, constituting a novel recoding translation mechanism for increasing the viral genome's coding capacity.

An extra SLVI is also present immediately upstream of the IGR IRESs of the honey bee viruses IAPV, KBV and ABPV IGR (Fig. 5.4) (Firth *et al.*, 2009). In fact, the stop codon for ORF1 is within the SLVI domain, hinting that translation of ORF1 may affect translation of ORF2. Indeed, mutations that disrupt SLVI formation moderately inhibit IGR IRES translation. This suggests both that the integrity of SLVI contributes to IGR IRES translation and that ORF1 and ORF2 translation may be coupled (Au *et al.*, 2018; Ren *et al.*, 2012). A recent study using an IAPV/CrPV chimeric virus clone showed that IAPV SLVI is important for virus infection (Au *et al.*, 2018).

IRES-mediated recoding is not restricted to the honey bee and fire ant dicistroviruses. The CrPV IGR IRES also directs +1 frame translation to produce a small 41 amino acid ORFx protein (Fig. 5.1B) (Kerr *et al.*, 2018b). However, unlike honey bee IGR IRESs, the CrPV IRES directs ribosome bypass, where a subset of ribosomes recruited to the CrPV IRES reposition downstream by 37 nucleotides to initiate translation of the +1 frame ORFx. Blocking ORFx expression by inserting a stop codon within the ORFx reading frame attenuates virus infection in fruit flies, thus demonstrating the biological relevance of CrPV ORFx in virus infection.

The function and specific role of ORFx in virus infection have yet to be determined. Mass spectrometry analysis has shown that ORFx is expressed in IAPV-infected honey bees and in CrPV-infected *Drosophila* cells, a strong indication that ORFx has a role in virus infection and/or pathogenesis (Ren *et al.*, 2012; Kerr *et al.*, 2018b). The ORFx of CrPV and honey bee dicistroviruses do not have any obvious sequence similarity, nor do they have any obvious homology to known proteins (Fig. 5.1B). However, CrPV ORFx has a predicted transmembrane domain and can associate with the ER membrane (Kerr *et al.*, 2018b). Although the CrPV ORFx importance to virus infection has been established (Kerr *et al.*, 2018b), an infectious IAPV clone is not available to test IAPV ORFx functionality via reverse genetics approaches. However, the recent development of a chimeric IAPV/CrPV infectious clone with CrPV IGR IRES swapped with IAPV IGR IRES may improve future research into the role of the IAPV IGR IRES and ORFx protein (Au *et al.*, 2018).

Ribosome heterogeneity and IGR IRES translation

Because of its streamlined mechanism, the IGR IRES has become a model for understanding more complex IRES mechanisms, including those of clinically relevant viruses such as *Human*

immunodeficiency virus (HIV) and *Hepatitis C virus* (HCV). Some of the conformational changes within the IGR IRES and ribosome resemble that of HCV IRES:ribosome interactions (Spahn *et al.*, 2004; Pisareva *et al.*, 2018), suggesting that these IRES-induced conformations are intrinsic to ribosome function. In a surprising twist, work with IGR IRES has led to investigations into insights of ribosome heterogeneity. As discussed, the IGR IRES contacts the ribosome directly; for example, SLV interacts with eS25 (Nishiyama *et al.*, 2003; Schüler *et al.*, 2006; Muhs *et al.*, 2011). Interestingly, depletion of some ribosomal proteins (e.g. eS25 and eS6) inhibits IGR IRES- but not cap-dependent translation, suggesting that distinct ribosomes can mediate translation of specific mRNAs (Landry *et al.*, 2009; Muhs *et al.*, 2011). These findings have led to research showing that the depletion of other ribosomal proteins not only impacts IGR IRES translation, but also that of *Hepatitis C virus* and cellular IRESs as well as ribosome shunting, indicating that ribosome heterogeneity may be an important factor in dictating translation of specific mRNAs (Cherry *et al.*, 2005; Hertz *et al.*, 2013; Carvajal *et al.*, 2016). Besides ribosomal proteins, IGR IRES has been linked to pseudouridylation in ribosomal RNA. Experimental depletion of pseudouridines led to ribosomes with reduced affinity for the IGR IRES that could not support IRES translation, despite having limited impact on overall cap-dependent translation (Jack *et al.*, 2011). These findings demonstrate that ribosome heterogeneity deriving from both post-translational and post-transcriptional modifications and from specialized functions of individual ribosomal components can affect the translation of specific mRNAs. The challenge is to identify the specific modifications and/or translation initiation complexes of importance. Clarification is still needed on whether specialized ribosomes are a major determinant in dicistrovirus infection.

The fact that dicistrovirus IGR IRES can bind directly to the conserved tRNA-binding core of the ribosome to direct translation could explain how this IRES can function across eukaryotes including yeast, insect and mammalian cells (Thompson *et al.*, 2001; Fernandez *et al.*, 2002). IRES can also bind to prokaryotic ribosomes and function in bacteria; however, the mechanism of 80S assembly is distinct from that in eukaryotic cells (Zhu *et al.*, 2011; Colussi *et al.*, 2014). The simplicity of the IGR IRES's direct recruitment of ribosomal machinery is a powerful model to understand ribosome function and dynamics. Furthermore, it provides insight into tRNA–ribosome interactions that mediate reading frame selection and maintenance, and highlights diverse strategies for directing viral protein synthesis.

5′UTR IRES

Despite the extensive characterization of the IGR IRES, studies of the 5′UTR IRES of dicistroviruses have been limited, partly due to the lack of obvious sequence and structural conservation (Jan, 2006). The 5′UTR IRESs range in length from 27 (SINV-1) to 964 (HiPV) nucleotides. This IRES mechanism has only been studied for the CrPV, RhPV and the dicistro-like Halastavi árva virus (HaIV) 5′UTR IRESs. Biochemical reconstitution methods using purified translation factors and ribosomes assembled on the IRES have shown that HalV and RhPV IRESs use a similar subset of factors (eIF1, eIF1A, eIF2 and eIF3) to promote AUG recognition by the preinitiation 40S complex (Woolaway *et al.*, 2001; Roberts and Groppelli, 2009; Abaeva *et al.*, 2016). RhPV IRES is strongly dependent on the RNA helicase eIF4A, but the HalV IRES is not. Both HaIV and RhPV use an unstructured region within the 5′UTR or near the AUG codon to recruit the 40S subunit. Once bound to the RhPV IRES, the 40S scans to locate the AUG codon. In contrast, binding of 40S to the HaIV IRES results in retrograde scanning of the ribosome to locate the AUG codon (Abaeva *et al.*, 2016). Interestingly, both HaIV and RhPV 5′UTR IRESs are functional across kingdoms; the HaIV and RhPV 5′UTR IRESs are functional in plant, mammalian and insect cell lysates. Thus, there may be common properties between some dicistrovirus 5′UTR IRESs, despite the differences in their sequences and structures.

In contrast to RhPV and HaIV, the 5′UTR IRES of CrPV does not use a scanning mechanism. Instead, it directly recruits the ribosome to the AUG codon (Gross *et al.*, 2017). RNA structural probing revealed several stem loops and a pseudoknot that are required for IRES translation. Based on these properties, the CrPV 5′UTR IRES mechanism

may be similar to that of the HCV IRES. Interestingly, both HCV and the related cripavirus DCV require the ribosomal protein RACK1 for 5′UTR IRES translation and virus infection (Majzoub *et al.*, 2014). The finding that RACK1 is required for dicistrovirus 5′UTR IRES further supports the idea that ribosome heterogeneity plays a role in IRES-mediated translation in dicistrovirus and other virus infections.

The different 5′UTR IRES mechanisms of dicistroviruses may have evolved distinct translation mechanisms for each host species. The 5′UTR IRES directs translation of the dicistrovirus ORF1, which encodes the non-structural proteins RdRp, helicase and protease, all of which are required for viral replication. Thus, each dicistrovirus 5′UTR IRES mechanism is optimized for each host species during infection in order to promote translation of the non-structural proteins.

During dicistrovirus infection, the structural proteins are produced in supramolar excess compared to non-structural proteins (Moore *et al.*, 1980; Wilson *et al.*, 2000b; Garrey *et al.*, 2010), largely due to the distinct mechanisms of the 5′UTR and IGR IRESs. The 5′UTR IRES is relatively weak, while the IGR IRES is stimulated as infection progresses (Wilson *et al.*, 2000a; Khong *et al.*, 2016). Translation from the 5′UTR IRES and IGR IRES is also temporally regulated during infection; the 5′UTR IRES is active first, followed by the IGR IRES (Khong *et al.*, 2016). IGR IRES translation is presumably delayed on infection to allow for the 5′UTR IRES to express the non-structural proteins needed for replication of the viral genome. Once sufficient viral replication is achieved, IGR IRES translation is stimulated to drive expression of the structural proteins required for viral packaging. Thus, the CrPV uses a dual IRES translational control strategy to ensure optimal temporal expression of viral non-structural proteins prior to synthesis of viral structural proteins, which coordinates the timing of viral replication and assembly during the dicistrovirus life cycle. The mechanisms that regulate the dual IRESs in dicistrovirus-infected cells remain to be investigated. However, host translational shut-off and recycling of ribosomes by Pelo, a homologue of the yeast Dom34, may contribute to this temporal regulation (Garrey *et al.*, 2010; Wu *et al.*, 2014).

Host responses to viral infection

Shut off of host protein synthesis

Dicistrovirus infection leads to a rapid shut off of host protein synthesis concomitant with a switch to viral IRES-dependent translation (Moore *et al.*, 1980, 1981; Wilson *et al.*, 2000b; Garrey *et al.*, 2010) (Table 5.1). Although these observations are primarily from CrPV- and DCV-infected *Drosophila* cells, it is likely that other dicistrovirus infections lead to host translation shut-off. Viruses may employ host translational shut off (1) to block induction of antiviral innate immune protein expression and (2) to increase the pool of available ribosomes for viral protein synthesis. It is also possible that the shut off of translation is a host response akin to a cellular stress response. In poliovirus infections, host translation shutdown is a viral strategy; the poliovirus 2A protease cleaves translation factor eIF4G (Gradi *et al.*, 1998).

During dicistrovirus infection of S2 cells by CrPV, the translation factor eIF2α is phosphorylated and interactions between eIF4E, cap binding protein, and eIF4G are inhibited (Garrey *et al.*, 2010). eIF2 is a trimeric complex of α, β, and γ subunits, which brings the initiator Met-tRNA$_i$ to the 40S pre-initiation complex at the 5'cap of mRNAs. Phosphorylation of eIF2α reduces the pool of available eIF2, leading to a decrease in translation. Phosphorylation of eIF2α could be a viral strategy to inhibit host translation, as neither the viral IGR IRES mechanism nor the initiator Met-tRNA$_i$ requires translation factors to promote ORF2 protein synthesis. However, blocking eIF2α phosphorylation in CrPV infected S2 cells by either depleting both eIF2α kinases in *Drosophila* (dPERK, dGCN2) or inducing the phosphatase dGADD34 still results in host translation inhibition and viral protein synthesis, suggesting that another translation factor is targeted (Garrey *et al.*, 2010; Khong *et al.*, 2016). The disruption of eIF4E–eIF4G interactions in CrPV-infected S2 cells does not involve the 4E-binding protein or *Drosophila* Cup, both known inhibitors of eIF4E activity. Whether targeting eIF4E–eIF4G interactions is the main determinant for host translation shut off under dicistrovirus infection has yet to be established. Other mechanisms may contribute to translation shut off. For example, IAPV infection of

honey bees results in ribosomal RNA fragmentation (Johnson *et al.*, 2009).

Antiviral responses

Dicistroviruses have served as excellent models to study fundamental virus–host interactions and antiviral responses in insects. We briefly review the main innate immune pathways in insects and the impact of these pathways on dicistrovirus infections using DCV and CrPV infections as a model. A more comprehensive and detailed overview of the insect immune responses is provided in Chapter 3.

Four main innate immune pathways exist in insects: Toll, IMD, JAK-STAT and RNA interference pathways (Kingsolver *et al.*, 2013). Initially identified from responses to fungal and bacterial infections, triggering of the Toll and IMD pathways activates NFκB-like transcription factors, Dif and Relish, respectively. In turn, activated Dif or Relish leads to transcription of immune genes for the antimicrobial peptides Drosomycin (Toll) and Diptericin (IMD). Activation of the JAK-STAT pathway responds to septic infection in *Drosophila* (Zeidler and Bausek, 2013). The JAK Kinase, Hopscotch protein and the STAT transcription factor (STAT92E), respond to activation of the Domeless receptor by three cytokines of the unpaired (UPD) family. Although these four pathways have all been implicated in response to dicistrovirus infection, the RNAi pathway is the main insect antiviral response (Chapter 3).

RNA interference

The RNA interference pathway is the major antiviral response in insects (Chapter 3). In virus-infected *Drosophila*, double stranded RNA (dsRNA) intermediates produced during replication are sensed by Dicer-2, a ribonuclease III enzyme. Dicer-2 processes the dsRNA into ≈21–23 nucleotide small interfering RNAs (siRNA). The siRNAs, in concert with the RNA-induced silencing complex (RISC), which contains Ago-2, surveils for target RNAs and induces cleavage (Wilson and Doudna, 2013). The importance of the RNAi pathway in antiviral insect responses has been established by multiple studies. *ago-2*- and *dcr-2*-deficient *Drosophila* are hypersensitive to CrPV and DCV infections (Galiana-Arnoux *et al.*, 2006; Kingsolver *et al.*, 2013; van Rij *et al.*, 2006). *ago2*-deficient flies showed a significant increase in viral RNA accumulation, a 1000-fold increase in viral titre, and an increased mortality rate.

Insects can mediate systemic immunity in response to virus infection. In *Drosophila*, mutant flies defective in the dsRNA update pathway (*egh*, *ninaC* or *CG4572*) are hypersensitive to DCV, suggesting that uninfected cells may participate in antiviral immunity (Saleh *et al.*, 2006, 2009). Systemic RNAi-based immunity may involve haemocytes, which take up dsRNA from infected cells to produce virus-derived complementary DNAs (vDNAs) through the action of endogenous transposon-encoded reverse transcriptase (Goic *et al.*, 2013; Lamiable *et al.*, 2016; Tassetto *et al.*, 2017). This vDNA is present in both linear and circular forms, with the circular vDNA (cVDNA) bearing homology to the defective viral genome (DVG) and serving as a template for vDNA and cVDNA (Poirier *et al.*, 2018). The vDNA serves as template for the synthesis of viral siRNA (vsRNA) which is then incorporated into and secreted in exosome-like vesicles that confer systemic RNAi antiviral immunity (Tassetto *et al.*, 2017). The systemic spread of dsRNA may be a virus-specific immune signal akin to the immune responses elicited by plants and nematodes.

Inhibitors of RNA interference

In support of the RNAi pathway as the major insect antiviral response, dicistroviruses encode a silencing suppressor (VSS) that dampens this immune response in *Drosophila*. For CrPV and DCV, these RNAi suppressors map to the 1A protein at the most amino terminus of ORF1 (Nayak *et al.*, 2010; van Rij *et al.*, 2006; Wang *et al.*, 2006) (Table 5.1). Expression of CrPV 1A and DCV 1A in *Drosophila* cells blocked double-stranded RNA induced gene silencing, resulting in cells that were more susceptible to virus infection (Nayak *et al.*, 2010; van Rij *et al.*, 2006). Interestingly, DCV 1A and CrPV 1A proteins inhibit RNA interference via distinct mechanisms.

DCV 1A, a 97-amino acid protein, inhibits RNAi by binding to dsRNA, thus blocking processing by Dcr-2 and siRNA loading into RISC (van Rij *et al.*, 2006). In contrast, CrPV 1A, a 166-amino-acid protein, shows no dsRNA binding affinity and does not prevent siRNA loading into RISC. Instead, CrPV 1A modulates RNAi by interacting directly with Ago-2 to suppress its cleavage

activation (Nayak *et al.*, 2010). Using biochemical and single molecule imaging approaches, it has been shown that CrPV 1A blocks the Ago-2 RISC complex from targeting the RNA (Watanabe *et al.*, 2017). In a recent report by Andino and colleagues (Nayak *et al.*, 2018), CrPV 1A was shown to contain a BC box domain, which is responsible for mediating polyubiquitination of Ago-2 that results in proteosomal degradation. This divergence in the mechanisms of RNAi inhibition between DCV and CrPV may reflect differences in viral strategies for pathogenesis and/or the viral life cycle. Indeed, DCV and CrPV have distinct tissue tropisms in infected fruit flies, and CrPV but not DCV causes cytopathic effects in infected cells (Chtarbanova *et al.*, 2014). DCV 1A and CrPV 1A do not show any significant sequence similarity (9.27% similarity) and the proteins do not have obvious homology to known proteins. Moreover, it is not known if other dicistrovirus 1A proteins have similar VSS functions. Further structural and biochemical analysis should shed light into these mechanisms. Finally, CrPV 1A has recently been shown to have roles besides inhibiting the RNAi antiviral response (Khong *et al.*, 2017). How these multiple functions of 1A interplay to promote infection is a promising avenue for investigation.

JAK-STAT pathway

A global transcriptome analysis of flies injected with DCV revealed activation of the Jak-STAT pathway (Dostert *et al.*, 2005; Deddouche *et al.*, 2008; Kemp *et al.*, 2013). Virus injection into flies deficient in hopscotch, the main Jak kinase in *Drosophila*, resulted in higher viral loads and increased mortality to CrPV and DCV (Dostert *et al.*, 2005; Kemp *et al.*, 2013). The most induced genes under infection are *vir-1* (virus-induced RNA) and *vago*. Although vir-1 expression under virus infection is dependent on hopscotch via the Jak-STAT pathway, deleting vir-1 or overexpressing vir-1 does not affect virus infection, suggesting that vir-1 does not have a direct role in innate immunity (Dostert *et al.*, 2005). On the other hand, *vago* deficient flies are more susceptible to DCV infections. Interestingly, the induction of vago is dependent on Dcr-2 (Deddouche *et al.*, 2008). This finding suggests that Dcr-2 not only senses and activates RNAi immunity but also triggers an inducible antiviral pathway. How Dcr-2 signals to activate downstream genes is not clear. In a more recent study, flies deficient in the histone H3 lysine 9 methyltransferase, G9a, are more susceptible to DCV infection, which is associated with hyperactivation of the JAK-STAT pathway, thus linking an epigenetic mark to antiviral signalling (Merkling *et al.*, 2015). From these transcriptome analyses, there is little overlap between the genes induced from DCV infection versus bacteria or fungal infection (Dostert *et al.*, 2005). Moreover, following gene expression from a panel of different insect viruses, Jak-STAT signalling is virus specific (Kemp *et al.*, 2013).

Toll pathway

The Toll pathway is a major determinant for resistance to DCV oral infection of *Drosophila* (Ferreira *et al.*, 2014). *toll*-deficient flies are more susceptible to DCV oral infection, leading to increased mortality and higher viral loads. By contrast, this resistance is not observed with DCV injection of fruit flies, which is the most common method for infecting *Drosophila* with dicistrovirus. DCV oral infection leads to Toll pathway activation, resulting in translocation of the NFκB transcription factor, Dorsal, primarily in the fat body. Interestingly, this study found similar tissue tropism of DCV for both oral or injection-mediated infection (Ferreira *et al.*, 2014). In contrast, DCV oral infection resulted in infection of the midgut epithelial cells, which could be due to differences in feeding protocols (Xu *et al.*, 2013; Ferreira *et al.*, 2014). The latter study found that the extracellular signal-regulated kinase (ERK) signalling pathway was important for gut epithelium resistance to viral infection. Thus, *Drosophila* likely uses multiple antiviral pathways.

IMD pathway

The IMD pathway also contributes to antiviral activity against dicistrovirus infection. Mutant flies that are IMD pathway deficient are more susceptible to CrPV infection, with infection resulting in higher viral loads (Costa *et al.*, 2009). Although this suggests that the IMD pathway is protective, CrPV infection does not activate the IMD pathway based on monitoring the expression of downstream antimicrobial peptides. The IMD pathway may therefore depend on the gut microbiota to protect against virus infection. DCV oral infection in mutant flies deficient in the IMD pathway displayed an increase in viral replication in the intestine, supporting this

assertion (Sansone *et al.*, 2015). Gut microbiota thus may activate the IMD signalling pathway to boost antiviral responses (Sansone *et al.*, 2015). A recent RNAi screen by Imler and colleagues (Goto *et al.*, 2018) showed that the kinase dIKKβ and the NFκB-like transcription factor Relish control virus infection through the induction of antiviral genes such as STING.

Other factors involved in antiviral response

Apart from the Toll, IMD, JAK-STAT and RNAi pathways, several other host factors play key roles in modulating virus infection. The gene *pastrel* (*pst*) has been shown to restrict CrPV and DCV infection in flies (Magwire *et al.*, 2012; Martins *et al.*, 2014). Its resistance activity seems to be dicistrovirus specific, as there is no significant association with susceptibility to other positive strand RNA viruses. The double-stranded RNA-binding protein DIP1 (Disconnected interacting protein), which participates in a wide range of cellular processes, plays an antiviral role in S2 cells (Zhang *et al.*, 2015). *dip1* mutant flies are hypersensitive to DCV infection. The exact role of DIP1 in innate immune response remains to be investigated.

Apoptosis

Evading or delaying apoptosis is a strategy used by many viruses to establish infection (Benedict *et al.*, 2002). DCV infection in fruit flies induces the expression of Damm, which is one of the seven caspases in *Drosophila*, suggesting a potential link between DCV infection and apoptosis (Dostert *et al.*, 2005). Furthermore, apoptosis-dependent phagocytosis that removes virus-infected cells is induced in DCV-injected flies (Nainu *et al.*, 2015). Apoptosis has been shown to plays only a minor role in CrPV and DCV infections (Lamiable *et al.*, 2016). Interestingly, an inhibitor of apoptosis protein (IAP) repeat domain has been identified at the N terminus of the TSV ORF1 replicase protein, which suggests that some dicistroviruses may modulate apoptotic pathways during infection (Mari *et al.*, 2002).

Stress granules

Stress granules (SG) are dynamic cytoplasmic aggregates of RNAs and proteins that assemble in response to translation inhibition (Beckham and Parker, 2008). The function and mechanism of SG formation remains poorly understood. During virus infections, SG assembly may act as a host antiviral response by sequestering either the translation components from the viral RNA or the viral RNA itself. Therefore, viruses have evolved distinct mechanisms to modulate stress granule formation to facilitate virus infection (Lloyd, 2013). For example, poliovirus inhibits stress granule formation via cleavage of G3BP, a key stress granule protein, by the virally encoded 3C protease (White *et al.*, 2007). In West Nile virus infected cells, the recruitment of key stress granule proteins TIA-1 and TIAR to the minus strand viral RNA for replication inhibit SG assembly (Emara and Brinton, 2007). In vertebrates, SGs can recruit and activate the antiviral factor, PKR, a kinase that phosphorylates eIF2α to block viral translation (Reineke and Lloyd, 2015). Host translation is shut off in CrPV- and DCV-infected *Drosophila* S2 cells which typically leads to SG formation; however, in these cells SG assembly is inhibited, suggesting that viral modulation of SG assembly is evolutionarily conserved and that SGs may play an antiviral role (Khong and Jan, 2011) (Table 5.1).

Besides inhibiting Ago-2 activity (Nayak *et al.*, 2010, 2018), the CrPV 1A protein can also inhibit stress granule formation, indicating that the 1A protein is multifunctional, modulating several host pathways to promote infection (Khong *et al.*, 2017) (Table 5.1). Expression of 1A in S2 cells can inhibit SG formation in cells challenged with a SG inducer (i.e. heat shock, arsenite). CrPV 1A expression also blocks transcription of a subset of genes. A specific arginine R146 within 1A is important for these functions, which are separate from the VSS RNAi silencing activity of 1A. This separation indicates that these functions of 1A can be uncoupled (Khong *et al.*, 2017). Expression of a mutant R146A CrPV 1A resulted in loss of its ability to block SG and transcription. Furthermore, a mutant CrPV virus containing R146A resulted in increased SG formation in infected cells and attenuation of mortality in fruit flies injected with virus (Khong *et al.*, 2017). Interestingly, the expression of wild-type CrPV 1A but not mutant R146A 1A in Hela cells blocked SG formation, suggesting that 1A interferes with a conserved pathway in SG formation (Khong *et al.*, 2017).

Heat shock response

Heat shock of cells is protective against CrPV and DCV infection (Cevallos and Sarnow, 2010; Merkling *et al.*, 2015). Both viral RNA and protein abundance are high in heat shocked infected cells, suggesting that heat shock does not affect viral protein synthesis or replication. In contrast, virion formation is blocked in heat shocked infected cells. Therefore, the heat shock response, a cellular mechanism preventing proteotoxicity, can act as an antiviral component in *Drosophila*. Analysis of *in vitro* and *in vivo* transcriptomes in *Drosophila* revealed a strong induction of heat shock response genes with DCV and CrPV infection (Merkling *et al.*, 2015). These responses may be activated by the accumulation of unfolded viral protein in the cytosol.

Wolbachia pipientis infection confers protection against RNA viruses

A significant proportion of the microbiota in animals can confer immunity to pathogen infection, either through direct competition or indirectly by stimulating innate immunity (Belkaid and Hand, 2014). *Wolbachia* are Gram-negative, vertically transmitted, obligatory, intracellular bacteria that infect a number of arthropods and nematodes. The endosymbiotic association of *Wolbachia pipientis* with *Drosophila* has been shown to delay CrPV and DCV accumulation and mortality by an unknown mechanism (Hedges *et al.*, 2008; Teixeira *et al.*, 2008). Many *Wolbachia* strains infect *Drosophila* species, but not all mediate antiviral protection (Osborne *et al.*, 2009). The evolutionary success of *Wolbachia* relies on the strong and efficient vertical maternal transfer, which involves localization to the posterior pole of the oocyte to ensure its incorporation in the next generation (Serbus and Sullivan, 2007). Infection with *Wolbachia* reduced CrPV and DCV replication and conferred protection against other RNA viruses; however, the induced resistance was not effective against DNA viruses. The mechanism by which *Wolbachia* limits RNA virus infection is unknown; however, it is likely that the antiviral action results from up-regulation of immune responses. Elevated oxidative stress through the activation of extracellular signal-regulated kinase pathway is potentially associated with antiviral protection against DCV in *Wolbachia*-infected flies (Wong *et al.*, 2015, 2016).

Pathogenesis

Dicistrovirus pathology ranges from subtle or no clear symptoms to highly pathogenic, resulting in rapid death. Dicistrovirus infections are often associated with paralysis of the hind legs. Dicistrovirus has a wide tissue tropism that may explain disease progression. DCV has been detected in the crop, midgut, fat body and testes of DCV-infected *Drosophila* (Ferreira *et al.*, 2014). In infected fruit flies, DCV infects the crop, a blind muscular food storage organ. This results in the inability of the crop to contract, leading to accumulation of food, which causes significant malnutrition as well as accumulation of toxic compounds and dehydration, eventually leading to death (Chtarbanova *et al.*, 2014). In contrast, CrPV infection leads to an increase in crop contractions in infected fruit flies, likely as a compensatory mechanism for the impaired function of muscle cells in the midgut (Chtarbanova *et al.*, 2014). CrPV and ALPV have been localized to insect brains and the central nervous system using immunostaining against CrPV (Chtarbanova *et al.*, 2014) and *in situ* hybridization against ALPV (Hatfill *et al.*, 1990). These findings may explain the paralytic symptoms that are common in many dicistrovirus infections (Hatfill *et al.*, 1990). Dicistrovirus infections induce not only neurological changes but also widespread behavioural changes. RhPV infection alters the sensitivity of aphids to chemical cues, affecting how these insects choose plants to feed on. Infected aphids were also found to be more sensitive to the alarm pheromone than uninfected insects (Ban *et al.*, 2008). These insects are more susceptible to attack by the predatory beetle *Coccinella septempunctata* and the parasitoid *Aphidius ervi*. ALPV and IAPV infections result in a range of behavioural defects affecting orientation and navigation (Williamson *et al.*, 1988). In addition, IAPV infections of honey bees result in a significant decrease in the homing ability of foragers and a lower response threshold to sucrose (Li *et al.*, 2013; Han *et al.*, 2015)

Some dicistrovirus infections can result in widespread persistent infections with little or no symptoms. DCV infections can persist in *Drosophila* cell lines (Wu *et al.*, 2010). DCV, among other viruses, can also persistently infect about one third of wild and laboratory *D. melanogaster* colonies (Plus *et al.*, 1975; Kapun *et al.*, 2010). These persistent infections can have unintentional effects

on studies using this genetic model organism (Wu *et al.*, 2010). DCV infections also alter the viability of offspring; DCV-infected fruit flies produce more eggs and offspring than uninfected flies, and direct infection of larvae speeds up development (Gomariz-Zilber *et al.*, 1995). Conversely, KBV and IAPV infected honey bees result in slower colony startup and offspring production (Meeus *et al.*, 2014). Overall, the pathogenesis of this family of viruses varies dramatically. The details of the molecular mechanisms underlying the progression of these viral diseases remain to be examined.

Honey bee disease

The recent decline in honey bee populations worldwide is of critical agricultural importance. Crops that are predominantly pollinated by honey bees have an estimated value of more than US $215 billion dollars worldwide, and approximately a third of all food products consumed by humans are pollinated by honey bees (Gallai *et al.*, 2009). Honey bee losses resulting from colony collapse are multifactorial, but viral infections are significant contributors to bee disease (Cox-Foster *et al.*, 2007; Williams *et al.*, 2010). Honey bees are host to at least five dicistroviruses including ABPV, KBV, BQCV, IAPV, and an isolate of cricket paralysis virus. The latter has been shown to be asymptomatic (Christian and Scotti, 1998). Bee viruses spread horizontally via salivary secretions, food reserves, and faeces, as well through *Varroa* mite infestations (Chen and Siede, 2007).

Direct protection from and treatment of viral infections have been attempted for honey bees. Feeding bees with dsRNAs (encoding IAPV genome fragments) induced RNA interference protection of bees from IAPV infection (Maori *et al.*, 2009). Unfortunately, subsequent large-scale testing of 160 bee hives using RNAi approaches did not significantly affect bee mortality or infection by IAPV (Hunter *et al.*, 2010). A subset of dicistroviruses encode proteins that have anti-RNAi activity (van Rij *et al.*, 2006; Nayak *et al.*, 2010), which may reduce the efficacy of RNAi therapeutic approaches to treat virus infected bees.

Taura syndrome

The dicistrovirus *Taura syndrome virus* (TSV) is the causative agent of Taura syndrome in penaeid shrimp (Mari *et al.*, 2002). Taura syndrome outbreaks have had a devastating effect on the shrimp industry in the Americas, with a negative economic impact of over US $2 billion during the peak of the virus outbreak (Lightner, 1999). Originally limited to the Americas, Taura syndrome has spread to Asia, with outbreaks in Taiwan, Thailand, Myanmar, China, Korea and Indonesia (Kiatpathomchai *et al.*, 2008). Mortality of TSV is extremely high, with rates between 75% and 80% in *P. vannamei* (Wilson and Doudna, 2013). Intriguingly, infectious TSV has been found in seagull faeces, potentially providing a passive vector for virus transmission (Lightner, 1999; Garza *et al.*, 2011). Currently, resistant stocks of shrimps, selected through selective breeding for resistance along with the adoption of biosecurity practices, have reduced TSV to the point where it is no longer considered a major threat in many shrimp farming regions (Maori *et al.*, 2009; Moss *et al.*, 2012). Although direct antiviral strategies to combat Taura syndrome are lacking, a previous study showed that administering nonspecific dsRNA to shrimp resulted in resistance to TSV, suggesting that broad antiviral immunity can be achieved in arthropods (Robalino *et al.*, 2004, 2007).

Biopesticides

Management of insect pests is an ongoing global agricultural challenge. The use of pesticides results in a US$40 billion increase in agricultural production in the United States (Pimentel, 2005; Oerke, 2005; Popp *et al.*, 2012). Owing to the global concern that current pesticides can be detrimental to non-target species, there is emerging potential for the use of dicistroviruses as biopesticides. Dicistroviruses are attractive viral vectors for pest management for several reasons. Some dicistroviruses specifically infect host insect pests that negatively affect agriculture, such as RhPV infection of aphids. Viruses such as CrPV have a wide host range, and thus could target diverse hosts (Christian and Scotti, 1996). Since the establishment of the first dicistrovirus infectious clone (Kerr *et al.*, 2015), the potential use of dicistroviruses as biopesticides and the ability to use genetic engineering to maximize the effectiveness of this biopesticide presents an emerging opportunity.

Many dicistroviruses are pathogens of insects that cause substantial losses in crop production,

resulting from insect feeding and from insect-mediated transmission of plant viruses. For example, the virus *Homalodisca coagulata virus* (HoCV-1) infects the glassy-winged sharpshooter (GWSS), an insect that feeds on over 100 plant species, and can devastate crops (Hunnicutt *et al.*, 2008). The GWSS also serves as a vector for *Xylella fastidiosa*, a bacterium that results in plant death and has caused several disease outbreaks over the years, including Pierce's disease of grapes (PD) and citrus variegated chlorosis (CVC) (Redak *et al.*, 2004). HoCV-1 could potentially be used as a control agent for GWSS populations to avoid these diseases without the use of chemical insecticides (Falk *et al.*, 1992). In another example, SINV-1 has been proposed for use as a biopesticide against the invasive red fire ant (*Solenopsis invicta*). The red fire ant has invaded over 138 million hectares in the USA, causing over US $6 billion in damage and control efforts annually (Valles, 2012). SINV-1 can only infect the genus *Solenopsis* and is vertically transmitted (Valles, 2012). However, a recent study has found that SINV-1 treatment results in higher survival rates than chemical treatment (Tufts *et al.*, 2014).

One of the most exciting possibilities for use of dicistroviruses as biopesticides is the ability of these viruses to potentially be expressed in plants, enabling the production of plants expressing these viruses as a means to control pests (Gordon and Waterhouse, 2006). However, attempts to express viruses in plants have been largely unsuccessful because of RNAi silencing of viruses in the plants (Gordon and Waterhouse, 2006). One method proposed to circumvent this problem is to use dicistrovirus-like particles to deliver either dsRNA against essential genes of the infected host or toxins against the host (Gordon and Waterhouse, 2006; Baum *et al.*, 2007; Bonning and Johnson, 2010; Bonning *et al.*, 2014). As proof of principal, a luteovirus plant virus coat protein can deliver a toxin to aphids as those aphids feed on plants expressing the viral coat protein-insect neurotoxin fusion (Bonning *et al.*, 2014). A similar strategy could be implemented using a dicistrovirus clone expressed in plants that would then be transmitted to insects feeding on the plant. Indeed, some dicistroviruses have been shown to use plants as vectors (RhPV) (Gildow and d'Arcy, 1988).

The lethality of some dicistroviruses also has potential for exploitation. Incapable of infecting mammals, these viruses could be used to control disease-carrying hosts. Chagas disease, a devastating illness that has infected between 8 to 11 million people in Latin America is caused by the parasite *Trypanosoma cruzi*, which is spread by the insect vector *Triatoma infestans. Triatoma virus* (TrV) could be deployed as a protective measure, as its infection of *T. cruzi* leads to >97% mortality (Muscio *et al.*, 1997). Moreover, TrV infection increases adherence of *T. cruzi* to intestinal cells, making the vector less likely to shed the pathogen (Marti *et al.*, 2017).

Conclusions and future perspectives

Dicistroviruses have provided a powerful model for studying fundamental viral IRES translation, ribosome functions, antiviral responses and innate immune responses. Indeed, dicistrovirus research has attracted researchers from a wide range of fields including ecologists, virologists, immunologists, biochemists and biophysicists, all of which have brought innovative approaches that have accelerated our understanding of dicistrovirus infection and associated mechanisms. Despite significant progress, there are still outstanding gaps in our knowledge.

Although dicistrovirus IRES translation has been extensively studied at the mechanistic level, the rest of the viral life cycle, including viral entry, replication and egress, is understudied. Many of the host proteins involved in mediating specific steps of the viral life cycle are unknown. For instance, the receptor for viral entry has not been identified, nor have the host proteins involved in replication steps such as membrane reorganization and formation of replication complexes at the 5′ and 3′ UTR. An initial direction for further research is to identify key elements/structures at the 5′ and 3′UTRs needed for replication. As discussed, the 5′UTR secondary structure has recently been modelled, which should further our understanding of replication and 5′UTR IRES translation (Gross *et al.*, 2017). There are still knowledge gaps even for the well-studied IGR IRES translation mechanism. The mechanism by which a subset of IGR IRESs mediate reading frame selection through RNA–ribosome interactions remains to be investigated. These details

would shed light into fundamental tRNA-ribosome functions. Furthermore, the emerging idea that ribosome heterogeneity contributes to the translation of specific IRES-containing RNAs, warrants detailed investigation. Most studies in this field have focused on artificially depleting ribosomal proteins or specific RNA modifications that can impact dicistrovirus IRES translation. It will be important to determine which post-translational or post-transcriptional modifications of the ribosome are important for specifying viral IRES-mediated IRES translation. In particular, host translation shut off on dicistrovirus infection requires further study. Understanding the host targets for translational shut off on infection could reveal important insights into the cellular conditions that allow viral IGR IRES translation, as well as the temporal regulation of the two IRESs within dicistroviruses. Advances in whole genome-wide knockout screens using CRISPR-Cas9 approaches should help identify the functional proteins involved in these processes. From the virus side, the predicted functions of the non-structural and structural proteins are based on sequence alignments; however, it is likely that some have moonlighting functions that modulate cellular processes to promote infection. For example, the surprising finding that the CrPV 1A protein has multiple functions highlights the need to study other dicistrovirus proteins. The development of the CrPV infectious clone, which has already provided a framework for reverse genetics to explore the functions of 1A and IGR IRES translation, should accelerate our discovery of the functions of these viral proteins.

Understanding of these fundamental virus host interactions should allow insights into the pathogenesis of dicistroviruses. The use of the genetically tractable model organism *Drosophila*, has paved the way in studying dicistrovirus tropism, antiviral responses and pathogenesis. The use of the CrPV infectious clone has similar potential to provide insights into these processes in infected fruit flies. Transcriptomics has provided key insights into the antiviral responses in dicistrovirus infected *Drosophila*. Incorporating advanced translatome and proteomic approaches, such as ribosome profiling and siLAC labelling, should enable a global view of the pathways modulated during infection, potentially pinpointing key factors that can elucidate pathogenesis. Understanding of these mechanisms could allow for identification of new targets and strategies to combat diseases caused by dicistrovirus infections, such as virus-induced honey bee disease. For example, the function of the +1 frame overlapping ORFx in a subset of dicistroviruses remains elusive. The strong evolutionary selection of ORFx indicates an important function in infection and/or pathogenesis. Because the IGR IRES +1 frame translation and ORFx are found in honey bee dicistroviruses, it is possible that this mechanism/ORFx may be an attractive antiviral target. In a different vein, the use of dicistroviruses as biopesticides could have a large impact on serious agricultural pests. The diversity of dicistroviruses and the wide host range of some of these viruses makes them particularly attractive for this application. Harnessing and exploiting unique dicistrovirus mechanisms will be key in developing viral-based biopesticides. Finally, the recent identification of hundreds of dicistro-like viruses is exciting, highlighting the ubiquity and prevalence of dicistroviruses and should provide a treasure for researchers in identifying potentially new viral mechanisms.

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References

Abaeva, I.S., Pestova, T.V., and Hellen, C.U. (2016). Attachment of ribosomal complexes and retrograde scanning during initiation on the Halastavi árva virus IRES. Nucleic Acids Res. *44*, 2362–2377. https://doi.org/10.1093/nar/gkw016

Abeyrathne, P.D., Koh, C.S., Grant, T., Grigorieff, N., and Korostelev, A.A. (2016). Ensemble cryo-EM uncovers inchworm-like translocation of a viral IRES through the ribosome. Elife *5*, e14874. https://doi.org/10.7554/eLife.14874

Agirre, J., Goret, G., LeGoff, M., Sánchez-Eugenia, R., Marti, G.A., Navaza, J., Guérin, D.M.A., and Neumann, E. (2013). Cryo-electron microscopy reconstructions of triatoma virus particles: a clue to unravel genome delivery and capsid disassembly. J. Gen. Virol. *94*, 1058–1068.

Au, H.H., and Jan, E. (2012). Insights into factorless translational initiation by the tRNA-like pseudoknot domain of a viral IRES. PLOS ONE *7*, e51477. https://doi.org/10.1371/journal.pone.0051477

Au, H.H., Cornilescu, G., Mouzakis, K.D., Ren, Q., Burke, J.E., Lee, S., Butcher, S.E., and Jan, E. (2015). Global shape mimicry of tRNA within a viral internal ribosome

entry site mediates translational reading frame selection. Proc. Natl. Acad. Sci. U.S.A. *112*, E6446–55. https://doi.org/10.1073/pnas.1512088112

Au, H.H.T., Elspass, V.M., and Jan, E. (2018). Functional insights into the adjacent stem-loop in honey bee Dicistroviruses that promotes internal ribosome entry site-mediated translation and viral infection. J. Virol. *92*, e01725–17.

Bailey, L., and Woods, R.D. (1977). Two more small RNA viruses from honey bees and further observations on sacbrood and acute bee-paralysis viruses. J Gen. Virol. *37*, 175-182.

Bailey, L., Gibbs, A.J., and Woods, R.D. (1963). Two viruses from adult honey bees (*APIS mellifera linnaeus*). Virology *21*, 390–395.

Bailey, L., Gibbs, A.J., and Woods, R.D. (1964). Sacbrood virus of the larval honey bee (*APIS mellifera linnaeus*). Virology *23*, 425–429.

Ban, L., Didon, A., Jonsson, L.M., Glinwood, R., and Delp, G. (2007). An improved detection method for the Rhopalosiphum padi virus (RhPV) allows monitoring of its presence in aphids and movement within plants. J. Virol. Methods *142*, 136–142.

Ban, L., Ahmed, E., Ninkovic, V., Delp, G., and Glinwood, R. (2008). Infection with an insect virus affects olfactory behaviour and interactions with host plant and natural enemies in an aphid. Ent. Exp. Applicata *127*, 108-117.

Baum, J.A., Bogaert, T., Clinton, W., Heck, G.R., Feldmann, P., Ilagan, O., Johnson, S., Plaetinck, G., Munyikwa, T., Pleau, M., *et al.* (2007). Control of coleopteran insect pests through RNA interference. Nat. Biotechnol. *25*, 1322–1326.

Beckham, C.J., and Parker, R. (2008). P bodies, stress granules, and viral life cycles. Cell Host Microbe *3*, 206–212. https://doi.org/10.1016/j.chom.2008.03.004

Belkaid, Y., and Hand, T.W. (2014). Role of the microbiota in immunity and inflammation. Cell *157*, 121–141. https://doi.org/10.1016/j.cell.2014.03.011

Benedict, C.A., Norris, P.S., and Ware, C.F. (2002). To kill or be killed: viral evasion of apoptosis. Nat. Immunol. *3*, 1013–1018. https://doi.org/10.1038/ni1102-1013

Bird, S.W., Maynard, N.D., Covert, M.W., and Kirkegaard, K. (2014). Nonlytic viral spread enhanced by autophagy components. Proc. Natl. Acad. Sci. U.S.A. *111*, 13081–13086. https://doi.org/10.1073/pnas.1401437111

Bonning, B.C., and Johnson, K.N. (2010). Dicistroviruses. In Insect Virology, S. Asgari and K.N. Johnson, eds. (Caister Academic Press, Norfolk), pp. 201–229.

Bonning, B.C., Pal, N., Liu, S., Wang, Z., Sivakumar, S., Dixon, P.M., King, G.F., and Miller, W.A. (2014). Toxin delivery by the coat protein of an aphid-vectored plant virus provides plant resistance to aphids. Nat. Biotechnol. *32*, 102–105. https://doi.org/10.1038/nbt.2753

Brown, J.D., and Ryan, M.D. (2010). Ribosome 'Skipping': 'Stop-Carry On' or 'StopGo' translation. In Recoding: Expansion of Decoding Rules Enriches Gene Expression, J.F. Atkins, and R.F. Gesteland, eds. (Springer, New York), pp. 101–121.

Bugaud, O., Barbier, N., Chommy, H., Fiszman, N., Le Gall, A., Dulin, D., Saguy, M., Westbrook, N., Perronet, K., and Namy, O. (2017). Kinetics of CrPV and HCV IRES-mediated eukaryotic translation using single-molecule fluorescence microscopy. RNA *23*, 1626–1635. https://doi.org/10.1261/rna.061523.117

Carvajal, F., Vallejos, M., Walters, B., Contreras, N., Hertz, M.I., Olivares, E., Cáceres, C.J., Pino, K., Letelier, A., Thompson, S.R., *et al.* (2016). Structural domains within the HIV-1 mRNA and the ribosomal protein S25 influence cap-independent translation initiation. FEBS J. *283*, 2508–2527. https://doi.org/10.1111/febs.13756

Cevallos, R.C., and Sarnow, P. (2005). Factor-independent assembly of elongation-competent ribosomes by an internal ribosome entry site located in an RNA virus that infects penaeid shrimp. J. Virol. *79*, 677–683.

Cevallos, R.C., and Sarnow, P. (2010). Temperature protects insect cells from infection by cricket paralysis virus. J. Virol. *84*, 1652–1655. https://doi.org/10.1128/JVI.01730-09

Chen, Y.P., and Siede, R. (2007). Honey bee viruses. Adv. Virus Res. *70*, 33–80.

Cherry, S., and Perrimon, N. (2004). Entry is a rate-limiting step for viral infection in a *Drosophila* melanogaster model of pathogenesis. Nat. Immunol. *5*, 81–87. https://doi.org/10.1038/ni1019

Cherry, S., Doukas, T., Armknecht, S., Whelan, S., Wang, H., Sarnow, P., and Perrimon, N. (2005). Genome-wide RNAi screen reveals a specific sensitivity of IRES-containing RNA viruses to host translation inhibition. Genes Dev. *19*, 445–452.

Cherry, S., Kunte, A., Wang, H., Coyne, C., Rawson, R.B., and Perrimon, N. (2006). COPI activity coupled with fatty acid biosynthesis is required for viral replication. PLOS Pathog. *2*, e102.

Christian, P.D., and Scotti, P.D. (1996). Biopesticides from small RNA viruses on insects: aspects of their in vitro production. In Invertebrate Cell Culture: Looking Toward The Twenty First Century, K. Maramorosch, and M.J. Loeb, eds. (San Francisco: Proceedings of the IX International Conference on Invertebrate Cell Culture Society for In Vitro Biology), pp. 73–81.

Christian, P.D., and Scotti, P.D. (1998). Picornalike viruses of insects. In The Insect Viruses, L.K. Miller, and L.A. Ball, eds. (Springer, Boston, MA), pp. 301–336.

Chtarbanova, S., Lamiable, O., Lee, K.Z., Galiana, D., Troxler, L., Meignin, C., Hetru, C., Hoffmann, J.A., Daeffler, L., and Imler, J.L. (2014). *Drosophila* C virus systemic infection leads to intestinal obstruction. J. Virol. *88*, 14057–14069. https://doi.org/10.1128/JVI.02320-14

Colussi, T.M., Costantino, D.A., Hammond, J.A., Ruehle, G.M., Nix, J.C., and Kieft, J.S. (2014). The structural basis of transfer RNA mimicry and conformational plasticity by a viral RNA. Nature *511*, 366–369. https://doi.org/10.1038/nature13378

Costa, A., Jan, E., Sarnow, P., and Schneider, D. (2009). The Imd pathway is involved in antiviral immune responses in *Drosophila*. PLOS ONE *4*, e7436. https://doi.org/10.1371/journal.pone.0007436

Costantino, D., and Kieft, J.S. (2005). A preformed compact ribosome-binding domain in the cricket paralysis-like virus IRES RNAs. RNA *11*, 332–343.

Costantino, D.A., Pfingsten, J.S., Rambo, R.P., and Kieft, J.S. (2008). tRNA-mRNA mimicry drives translation initiation from a viral IRES. Nat. Struct. Mol. Biol. *15*, 57–64.

Cox-Foster, D.L., Conlan, S., Holmes, E.C., Palacios, G., Evans, J.D., Moran, N.A., Quan, P.L., Briese, T., Hornig, M., Geiser, D.M., *et al.* (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. Science *318*, 283–287.

D'Arcy, C.J., Burnett, P.A., Hewings, A.D., and Goodman, R.M. (1981a). Purification and characterization of a virus from the aphid Rhopalosiphum padi. Virology *112*, 346–349.

D'Arcy, C.J., Burnett, P.A., and Hewings, A.D. (1981b). Detection, biological effects, and transmission of a virus of the aphid Rhopalosiphum padi. Virology *114*, 268–272.

Deddouche, S., Matt, N., Budd, A., Mueller, S., Kemp, C., Galiana-Arnoux, D., Dostert, C., Antoniewski, C., Hoffmann, J.A., and Imler, J.L. (2008). The DExD/H-box helicase Dicer-2 mediates the induction of antiviral activity in *drosophila*. Nat. Immunol. *9*, 1425–1432. https://doi.org/10.1038/ni.1664

Donnelly, M.L., Hughes, L.E., Luke, G., Mendoza, H., ten Dam, E., Gani, D., and Ryan, M.D. (2001). The 'cleavage' activities of foot-and-mouth disease virus 2A site-directed mutants and naturally occurring '2A-like' sequences. J. Gen. Virol. *82*, 1027–1041.

Dostert, C., Jouanguy, E., Irving, P., Troxler, L., Galiana-Arnoux, D., Hetru, C., Hoffmann, J.A., and Imler, J.L. (2005). The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of *drosophila*. Nat. Immunol. *6*, 946–953.

Emara, M.M., and Brinton, M.A. (2007). Interaction of TIA-1/TIAR with West Nile and dengue virus products in infected cells interferes with stress granule formation and processing body assembly. Proc. Natl. Acad. Sci. U.S.A. *104*, 9041–9046.

Falk, M.M., Sobrino, F., and Beck, E. (1992). VPg gene amplification correlates with infective particle formation in foot-and-mouth disease virus. J. Virol. *66*, 2251–2260.

Feng, Z., Hensley, L., McKnight, K.L., Hu, F., Madden, V., Ping, L., Jeong, S.H., Walker, C., Lanford, R.E., and Lemon, S.M. (2013). A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. Nature *496*, 367–371. https://doi.org/10.1038/nature12029

Fernández, I.S., Bai, X.C., Murshudov, G., Scheres, S.H., and Ramakrishnan, V. (2014). Initiation of translation by cricket paralysis virus IRES requires its translocation in the ribosome. Cell *157*, 823–831. https://doi.org/10.1016/j.cell.2014.04.015

Fernandez, J., Yaman, I., Sarnow, P., Snider, M.D., and Hatzoglou, M. (2002). Regulation of internal ribosomal entry site-mediated translation by phosphorylation of the translation initiation factor eIF2alpha. J. Biol. Chem. *277*, 19198–19205. https://doi.org/10.1074/jbc.M201052200

Ferreira, Á.G., Naylor, H., Esteves, S.S., Pais, I.S., Martins, N.E., and Teixeira, L. (2014). The Toll-dorsal pathway is required for resistance to viral oral infection in *Drosophila*. PLOS Pathog. *10*, e1004507. https://doi.org/10.1371/journal.ppat.1004507

Firth, A.E., Wang, Q.S., Jan, E., and Atkins, J.F. (2009). Bioinformatic evidence for a stem-loop structure 5'-adjacent to the IGR-IRES and for an overlapping gene in the bee paralysis dicistroviruses. Virol. J. *6*, 193. https://doi.org/10.1186/1743-422X-6-193

Galiana-Arnoux, D., Dostert, C., Schneemann, A., Hoffmann, J.A., and Imler, J.L. (2006). Essential function in vivo for Dicer-2 in host defense against RNA viruses in *Drosophila*. Nat. Immunol. *7*, 590–597.

Gallai, N., Salles, J.-M., Settele, J., and Vaissière, B.E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecol. Econ. *68*, 810–821.

Garrey, J.L., Lee, Y.Y., Au, H.H., Bushell, M., and Jan, E. (2010). Host and viral translational mechanisms during cricket paralysis virus infection. J. Virol. *84*, 1124–1138. https://doi.org/10.1128/JVI.02006-09

Garza, J.R., Hasson, K.W., Poulos, B.T., Redman, R.M., White, B.L., and Lightner, D.V. (2011). Demonstration of infectious taura syndrome virus in the feces of seagulls collected during an epizootic in Texas. J. Aquat. Anim. Health *9*, 156–159.

Gildow, F., and D'Arcy, C.J.P. (1988). Barley and oats as reservoirs for an aphid virus and the influence on barley yellow dwarf virus transmission. Phytopathology *78*, 811–816.

Goic, B., Vodovar, N., Mondotte, J.A., Monot, C., Frangeul, L., Blanc, H., Gausson, V., Vera-Otarola, J., Cristofari, G., and Saleh, M.C. (2013). RNA-mediated interference and reverse transcription control the persistence of RNA viruses in the insect model *Drosophila*. Nat. Immunol. *14*, 396–403. https://doi.org/10.1038/ni.2542

Gomariz-Zilber, E., Poras, M., and Thomas-Orillard, M. (1995). *Drosophila* C virus: experimental study of infectious yields and underlying pathology in *Drosophila* melanogaster laboratory populations. J. Invertebr. Pathol. *65*, 243–247.

Goodfellow, I. (2011). The genome-linked protein VPg of vertebrate viruses - a multifaceted protein. Curr. Opin. Virol. *1*, 355–362. https://doi.org/10.1016/j.coviro.2011.09.003

Gordon, K.H., and Waterhouse, P.M. (2006). Small RNA viruses of insects: expression in plants and RNA silencing. Adv. Virus Res. *68*, 459–502.

Goto, A., Okado, K., Martins, N., Cai, H., Barbier, V., Lamiable, O., Troxler, L., Santiago, E., Kuhn, L., Paik, D., *et al.* (2018). The Kinase IKKβ Regulates a STING- and NF-κB-Dependent Antiviral Response Pathway in *Drosophila*. Immunity *49*, 225–234.e4.

Gradi, A., Svitkin, Y.V., Imataka, H., and Sonenberg, N. (1998). Proteolysis of human eukaryotic translation initiation factor eIF4GII, but not eIF4GI, coincides with the shutoff of host protein synthesis after poliovirus infection. Proc. Natl. Acad. Sci. U.S.A. *95*, 11089–11094.

Gross, L., Vicens, Q., Einhorn, E., Noireterre, A., Schaeffer, L., Kuhn, L., Imler, J.L., Eriani, G., Meignin, C., and Martin, F. (2017). The IRES5'UTR of the dicistrovirus cricket paralysis virus is a type III IRES containing an essential pseudoknot structure. Nucleic Acids Res. *45*, 8993–9004. https://doi.org/10.1093/nar/gkx622

Han, Y., van Oers, M.M., van Houte, S., and Ros, V.I.D. (2015). Virus-induced behavioural changes in insects. In Host Manipulations by Parasites and Viruses, H. Mehlhorn, ed. (Springer International Publishing, Cham), pp. 149–174.

Hasson, K., Lightner, D., Poulos, B., Redman, R., White, B., Brock, J., and Bonami, J. (1995). Taura syndrome in Penaeus vannamei: demonstration of a viral etiology. J. Dis. Aquat. Organ. *23*, 115–126.

Hatakeyama, Y., Shibuya, N., Nishiyama, T., and Nakashima, N. (2004). Structural variant of the intergenic internal ribosome entry site elements in dicistroviruses and computational search for their counterparts. RNA *10*, 779–786.

Hatfill, S.J., Williamson, C., Kirby, R., and von Wechmar, M.B. (1990). Identification and localization of aphid lethal paralysis virus particles in thin tissue sections of the Rhopalosiphum padi aphid by in situ nucleic acid hybridization. J. Invertebr. Pathol. *55*, 265–271.

Hedges, L.M., Brownlie, J.C., O'Neill, S.L., and Johnson, K.N. (2008). Wolbachia and virus protection in insects. Science *322*, 702. https://doi.org/10.1126/science.1162418

Hertz, M.I., and Thompson, S.R. (2011). In vivo functional analysis of the Dicistroviridae intergenic region internal ribosome entry sites. Nucleic Acids Res. *39*, 7276–7288. https://doi.org/10.1093/nar/gkr427

Hertz, M.I., Landry, D.M., Willis, A.E., Luo, G., and Thompson, S.R. (2013). Ribosomal protein S25 dependency reveals a common mechanism for diverse internal ribosome entry sites and ribosome shunting. Mol. Cell. Biol. *33*, 1016–1026. https://doi.org/10.1128/MCB.00879-12

Hinnebusch, A.G., Ivanov, I.P., and Sonenberg, N. (2016). Translational control by 5′-untranslated regions of eukaryotic mRNAs. Science *352*, 1413–1416. https://doi.org/10.1126/science.aad9868

Hunnicutt, L.E., Hunter, W.B., Cave, R.D., Powell, C.A., and Mozoruk, J.J. (2006). Genome sequence and molecular characterization of Homalodisca coagulata virus-1, a novel virus discovered in the glassy-winged sharpshooter (Hemiptera: Cicadellidae). Virology *350*, 67–78.

Hunnicutt, L.E., Mozoruk, J., Hunter, W.B., Crosslin, J.M., Cave, R.D., and Powell, C.A. (2008). Prevalence and natural host range of Homalodisca coagulata virus-1 (HoCV-1). Arch. Virol. *153*, 61–67. https://doi.org/10.1007/s00705-007-1066-2

Hunter, W., Ellis, J., Vanengelsdorp, D., Hayes, J., Westervelt, D., Glick, E., Williams, M., Sela, I., Maori, E., Pettis, J., *et al.* (2010). Large-scale field application of RNAi technology reducing Israeli acute paralysis virus disease in honey bees (Apis mellifera, Hymenoptera: Apidae). PLOS Pathog. *6*, e1001160. https://doi.org/10.1371/journal.ppat.1001160

Jack, K., Bellodi, C., Landry, D.M., Niederer, R.O., Meskauskas, A., Musalgaonkar, S., Kopmar, N., Krasnykh, O., Dean, A.M., Thompson, S.R., *et al.* (2011). rRNA pseudouridylation defects affect ribosomal ligand binding and translational fidelity from yeast to human cells. Mol. Cell *44*, 660–666. https://doi.org/10.1016/j.molcel.2011.09.017

Jackson, R.J., Hellen, C.U., and Pestova, T.V. (2010). The mechanism of eukaryotic translation initiation and principles of its regulation. Nat. Rev. Mol. Cell Biol. *11*, 113–127. https://doi.org/10.1038/nrm2838

Jan, E. (2006). Divergent IRES elements in invertebrates. Virus Res. *119*, 16–28.

Jan, E., and Sarnow, P. (2002). Factorless ribosome assembly on the internal ribosome entry site of cricket paralysis virus. J. Mol. Biol. *324*, 889–902.

Jan, E., Kinzy, T.G., and Sarnow, P. (2003). Divergent tRNA-like element supports initiation, elongation, and termination of protein biosynthesis. Proc. Natl. Acad. Sci. U.S.A. *100*, 15410–15415. https://doi.org/10.1073/pnas.2535183100

Jan, E., Mohr, I., and Walsh, D. (2016). A cap-to-tail guide to mRNA translation strategies in virus-infected cells. Annu. Rev. Virol. *3*, 283–307. https://doi.org/10.1146/annurev-virology-100114-055014

Jang, C.J., and Jan, E. (2010). Modular domains of the Dicistroviridae intergenic internal ribosome entry site. RNA *16*, 1182–1195. https://doi.org/10.1261/rna.2044610

Jang, C.J., Lo, M.C., and Jan, E. (2009). Conserved element of the dicistrovirus IGR IRES that mimics an E-site tRNA/ribosome interaction mediates multiple functions. J. Mol. Biol. *387*, 42–58. https://doi.org/10.1016/j.jmb.2009.01.042

Johnson, R.M., Evans, J.D., Robinson, G.E., and Berenbaum, M.R. (2009). Changes in transcript abundance relating to colony collapse disorder in honey bees (Apis mellifera). Proc. Natl. Acad. Sci. U.S.A. *106*, 14790–14795. https://doi.org/10.1073/pnas.0906970106

Jousset, F.X., Plus, N., Croizier, G., and Thomas, M. (1972). Existence chez *Drosophila* de deux groupes de Picornavirus de propriétés sérologiques et biologiques différentes. C. R. Acad. Sci. Hebd. Seances Acad. Sci. D. *275*, 3043–3046.

Kamoshita, N., Nomoto, A., and RajBhandary, U.L. (2009). Translation initiation from the ribosomal A site or the P site, dependent on the conformation of RNA pseudoknot I in dicistrovirus RNAs. Mol. Cell *35*, 181–190. https://doi.org/10.1016/j.molcel.2009.05.024

Kanamori, Y., and Nakashima, N. (2001). A tertiary structure model of the internal ribosome entry site (IRES) for methionine-independent initiation of translation. RNA *7*, 266–274.

Kapun, M., Nolte, V., Flatt, T., and Schlötterer, C. (2010). Host range and specificity of the *Drosophila* C virus. PLOS ONE *5*, e12421. https://doi.org/10.1371/journal.pone.0012421

Kemp, C., Mueller, S., Goto, A., Barbier, V., Paro, S., Bonnay, F., Dostert, C., Troxler, L., Hetru, C., Meignin, C., *et al.* (2013). Broad RNA interference-mediated antiviral immunity and virus-specific inducible responses in *Drosophila*. J. Immunol. *190*, 650–658. https://doi.org/10.4049/jimmunol.1102486

Kerr, C.H., Wang, Q.S., Keatings, K., Khong, A., Allan, D., Yip, C.K., Foster, L.J., and Jan, E. (2015). The 5′ untranslated region of a novel infectious molecular clone of the dicistrovirus cricket paralysis virus modulates infection. J. Virol. *89*, 5919–5934. https://doi.org/10.1128/JVI.00463-15

Kerr, C.H., Ma, Z.W., Jang, C.J., Thompson, S.R., and Jan, E. (2016). Molecular analysis of the factorless internal ribosome entry site in Cricket Paralysis virus infection. Sci. Rep. *6*, 37319. https://doi.org/10.1038/srep37319

Kerr, C.H., Dalwadi, U., Scott, N.E., Yip, C.K., Foster, L.J., and Jan, E. (2018a). Transmission of Cricket paralysis virus via exosome-like vesicles during infection of

Drosophila cells. Sci. Rep. *8*, 17353. https://doi.org/10.1038/s41598-018-35717-5

Kerr, C.H., Wang, Q.S., Moon, K.M., Keatings, K., Allan, D.W., Foster, L.J., and Jan, E. (2018b). IRES-dependent ribosome repositioning directs translation of a +1 overlapping ORF that enhances viral infection. Nucleic Acids Res. *46*, 11952–11967. https://doi.org/10.1093/nar/gky1121

Khong, A., and Jan, E. (2011). Modulation of stress granules and P bodies during dicistrovirus infection. J. Virol. *85*, 1439–1451. https://doi.org/10.1128/JVI.02220-10

Khong, A., Bonderoff, J.M., Spriggs, R.V., Tammpere, E., Kerr, C.H., Jackson, T.J., Willis, A.E., and Jan, E. (2016). Temporal regulation of distinct internal ribosome entry sites of the Dicistroviridae cricket paralysis virus. Viruses *8*, E25. https://doi.org/10.3390/v8010025

Khong, A., Kerr, C.H., Yeung, C.H., Keatings, K., Nayak, A., Allan, D.W., and Jan, E. (2017). Disruption of stress granule formation by the multifunctional cricket paralysis virus 1A protein. J. Virol. *91*, e01779–16.

Kiatpathomchai, W., Jaroenram, W., Arunrut, N., Gangnonngiw, W., Boonyawiwat, V., and Sithigorngul, P. (2008). Experimental infections reveal that common Thai crustaceans are potential carriers for spread of exotic Taura syndrome virus. Dis. Aquat. Org. *79*, 183–190. https://doi.org/10.3354/dao01903

King, L.A., and Moore, N.F. (1988). Evidence for the presence of a genome-linked protein in two insect picornaviruses, cricket paralysis and *Drosophila* C viruses. FEMS Microbiol. Lett. *50*, 41–44.

Kingsolver, M.B., Huang, Z., and Hardy, R.W. (2013). Insect antiviral innate immunity: pathways, effectors, and connections. J. Mol. Biol. *425*, 4921–4936. https://doi.org/10.1016/j.jmb.2013.10.006

Koh, C.S., Brilot, A.F., Grigorieff, N., and Korostelev, A.A. (2014). Taura syndrome virus IRES initiates translation by binding its tRNA-mRNA-like structural element in the ribosomal decoding center. Proc. Natl. Acad. Sci. U.S.A. *111*, 9139–9144. https://doi.org/10.1073/pnas.1406335111

Koonin, E.V., Wolf, Y.I., Nagasaki, K., and Dolja, V.V. (2008). The Big Bang of picorna-like virus evolution antedates the radiation of eukaryotic supergroups. Nat. Rev. Microbiol. *6*, 925–939. https://doi.org/10.1038/nrmicro2030

Lamiable, O., Arnold, J., de Faria, I.J.D.S., Olmo, R.P., Bergami, F., Meignin, C., Hoffmann, J.A., Marques, J.T., and Imler, J.L. (2016). Analysis of the contribution of hemocytes and autophagy to *Drosophila* antiviral immunity. J. Virol. *90*, 5415–5426. https://doi.org/10.1128/JVI.00238-16

Landry, D.M., Hertz, M.I., and Thompson, S.R. (2009). RPS25 is essential for translation initiation by the Dicistroviridae and hepatitis C viral IRESs. Genes Dev. *23*, 2753–2764. https://doi.org/10.1101/gad.1832209

Leppek, K., Das, R., and Barna, M. (2018). Functional 5' UTR mRNA structures in eukaryotic translation regulation and how to find them. Nat. Rev. Mol. Cell Biol. *19*, 158–174. https://doi.org/10.1038/nrm.2017.103

Letunic, I., and Bork, P. (2016). Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Res. *44*, W242–5. https://doi.org/10.1093/nar/gkw290

Li, Z., Chen, Y., Zhang, S., Chen, S., Li, W., Yan, L., Shi, L., Wu, L., Sohr, A., and Su, S. (2013). Viral infection affects sucrose responsiveness and homing ability of forager honey bees, Apis mellifera L. PLOS ONE *8*, e77354. https://doi.org/10.1371/journal.pone.0077354

Lightner, D.V. (1999). The Penaeid shrimp viruses TSV, IHHNV, WSSV, and YHV. J. Appl. Aquaculture *9*, 27–52.

Lloyd, R.E. (2013). Regulation of stress granules and P-bodies during RNA virus infection. Wiley Interdiscip. Rev. RNA *4*, 317–331. https://doi.org/10.1002/wrna.1162

Luke, G.A., de Felipe, P., Lukashev, A., Kallioinen, S.E., Bruno, E.A., and Ryan, M.D. (2008). Occurrence, function and evolutionary origins of '2A-like' sequences in virus genomes. J. Gen. Virol. *89*, 1036–1042.

Magwire, M.M., Fabian, D.K., Schweyen, H., Cao, C., Longdon, B., Bayer, F., and Jiggins, F.M. (2012). Genome-wide association studies reveal a simple genetic basis of resistance to naturally coevolving viruses in *Drosophila* melanogaster. PLOS Genet. *8*, e1003057. https://doi.org/10.1371/journal.pgen.1003057

Mailliot, J., and Martin, F. (2018). Viral internal ribosomal entry sites: four classes for one goal. Wiley Interdiscip. Rev. RNA *9*. https://doi.org/10.1002/wrna.1458

Majzoub, K., Hafirassou, M.L., Meignin, C., Goto, A., Marzi, S., Fedorova, A., Verdier, Y., Vinh, J., Hoffmann, J.A., Martin, F., *et al.* (2014). RACK1 controls IRES-mediated translation of viruses. Cell *159*, 1086–1095.

Maori, E., Lavi, S., Mozes-Koch, R., Gantman, Y., Peretz, Y., Edelbaum, O., Tanne, E., and Sela, I. (2007). Isolation and characterization of Israeli acute paralysis virus, a dicistrovirus affecting honeybees in Israel: evidence for diversity due to intra- and inter-species recombination. J. Gen. Virol. *88*, 3428–3438.

Maori, E., Paldi, N., Shafir, S., Kalev, H., Tsur, E., Glick, E., and Sela, I. (2009). IAPV, a bee-affecting virus associated with Colony Collapse Disorder can be silenced by dsRNA ingestion. Insect Mol. Biol. *18*, 55–60. https://doi.org/10.1111/j.1365-2583.2009.00847.x

Mari, J., Poulos, B.T., Lightner, D.V., and Bonami, J.R. (2002). Shrimp Taura syndrome virus: genomic characterization and similarity with members of the genus Cricket paralysis-like viruses. J. Gen. Virol. *83*, 915–926.

Marti, G.A., Ragone, P., Balsalobre, A., Ceccarelli, S., Susevich, M.L., Diosque, P., Echeverría, M.G., and Rabinovich, J.E. (2017). Can Triatoma virus inhibit infection of Trypanosoma cruzi (Chagas, 1909) in Triatoma infestans (Klug)? A cross infection and co-infection study. J. Invertebr. Pathol. *150*, 101–105.

Martins, N.E., Faria, V.G., Nolte, V., Schlötterer, C., Teixeira, L., Sucena, É., and Magalhães, S. (2014). Host adaptation to viruses relies on few genes with different cross-resistance properties. Proc. Natl. Acad. Sci. U.S.A. *111*, 5938–5943. https://doi.org/10.1073/pnas.1400378111

Masoumi, A., Hanzlik, T.N., and Christian, P.D. (2003). Functionality of the 5'- and intergenic IRES elements of cricket paralysis virus in a range of insect cell lines, and its relationship with viral activities. Virus Res. *94*, 113–120.

Meeus, I., de Miranda, J.R., de Graaf, D.C., Wäckers, F., and Smagghe, G. (2014). Effect of oral infection with Kashmir bee virus and Israeli acute paralysis virus on bumblebee (Bombus terrestris) reproductive success. J. Invertebr. Pathol. *121*, 64–69. https://doi.org/10.1016/j.jip.2014.06.011

Merkling, S.H., Bronkhorst, A.W., Kramer, J.M., Overheul, G.J., Schenck, A., and Van Rij, R.P. (2015). The epigenetic regulator G9a mediates tolerance to RNA virus infection in *Drosophila*. PLOS Pathog. *11*, e1004692. https://doi.org/10.1371/journal.ppat.1004692

Moore, N.F., Kearns, A., and Pullin, J.S. (1980). Characterization of cricket paralysis virus-induced polypeptides in *Drosophila* cells. J. Virol. *33*, 1–9.

Moore, N.F., Reavy, B., Pullin, J.S., and Plus, N. (1981). The polypeptides induced in *Drosophila* cells by *Drosophila C* virus (strain Ouarzazate). Virology *112*, 411–416.

Moss, S.M., Moss, D.R., Arce, S.M., Lightner, D.V., and Lotz, J.M. (2012). The role of selective breeding and biosecurity in the prevention of disease in penaeid shrimp aquaculture. J. Invertebr. Pathol. *110*, 247–250. https://doi.org/10.1016/j.jip.2012.01.013

Muhs, M., Yamamoto, H., Ismer, J., Takaku, H., Nashimoto, M., Uchiumi, T., Nakashima, N., Mielke, T., Hildebrand, P.W., Nierhaus, K.H., *et al.* (2011). Structural basis for the binding of IRES RNAs to the head of the ribosomal 40S subunit. Nucleic Acids Res. *39*, 5264–5275. https://doi.org/10.1093/nar/gkr114

Muhs, M., Hilal, T., Mielke, T., Skabkin, M.A., Sanbonmatsu, K.Y., Pestova, T.V., and Spahn, C.M. (2015). Cryo-EM of ribosomal 80S complexes with termination factors reveals the translocated cricket paralysis virus IRES. Mol. Cell *57*, 422–432. https://doi.org/10.1016/j.molcel.2014.12.016

Mullapudi, E., Přidal, A., Pálková, L., de Miranda, J.R., and Plevka, P. (2016). Virion structure of Israeli acute bee paralysis virus. J. Virol. *90*, 8150–8159. https://doi.org/10.1128/JVI.00854-16

Murray, J., Savva, C.G., Shin, B.S., Dever, T.E., Ramakrishnan, V., and Fernández, I.S. (2016). Structural characterization of ribosome recruitment and translocation by type IV IRES. Elife *5*, e13567. https://doi.org/10.7554/eLife.13567

Muscio, O.A., LaTorre, J.L., and Scodeller, E.A. (1987). Small nonoccluded viruses from triatomine bug Triatoma infestans (Hemiptera: Reduviidae). J. Invertebr. Pathol. *49*, 218–220.

Muscio, O.A., La Torre, J., Bonder, M.A., and Scodeller, E.A. (1997). Triatoma virus pathogenicity in laboratory colonies of Triatoma infestans (Hemiptera:Reduviidae). J. Med. Entomol. *34*, 253–256.

Muscio, O.A., Bonder, M.A., La Torre, J.L., and Scodeller, E.A. (2000). Horizontal transmission of triatoma virus through the fecal-oral route in Triatoma infestans (Hemiptera: Triatomidae). J. Med. Entomol. *37*, 271–275.

Nainu, F., Tanaka, Y., Shiratsuchi, A., and Nakanishi, Y. (2015). Protection of insects against viral infection by apoptosis-dependent phagocytosis. J. Immunol. *195*, 5696–5706. https://doi.org/10.4049/jimmunol.1500613

Nakashima, N., and Ishibashi, J. (2010). Identification of the 3C-protease-mediated 2A/2B and 2B/2C cleavage sites in the nonstructural polyprotein precursor of a Dicistrovirus lacking the NPGP motif. Arch. Virol. *155*, 1477–1482. https://doi.org/10.1007/s00705-010-0723-z

Nakashima, N., and Nakamura, Y. (2008). Cleavage sites of the 'P3 region' in the nonstructural polyprotein precursor of a dicistrovirus. Arch. Virol. *153*, 1955–1960. https://doi.org/10.1007/s00705-008-0208-5

Nakashima, N., and Shibuya, N. (2006). Multiple coding sequences for the genome-linked virus protein (VPg) in dicistroviruses. J. Invertebr. Pathol. *92*, 100–104.

Nakashima, N., and Uchiumi, T. (2009). Functional analysis of structural motifs in dicistroviruses. Virus Res. *139*, 137–147. https://doi.org/10.1016/j.virusres.2008.06.006

Nakashima, N., Sasaki, J., Tsuda, K., Yasunaga, C., and Noda, H. (1998). Properties of a new picorna-like virus of the brown-winged green bug, plautia stali J. Invertebr. Pathol. *71*, 151–158.

Nayak, A., Berry, B., Tassetto, M., Kunitomi, M., Acevedo, A., Deng, C., Krutchinsky, A., Gross, J., Antoniewski, C., and Andino, R. (2010). Cricket paralysis virus antagonizes Argonaute 2 to modulate antiviral defense in *Drosophila*. Nat. Struct. Mol. Biol. *17*, 547–554. https://doi.org/10.1038/nsmb.1810

Nayak, A., Kim, D.Y., Trnka, M.J., Kerr, C.H., Lidsky, P.V., Stanley, D.J., Rivera, B.M., Li, K.H., Burlingame, A.L., Jan, E., *et al.* (2018). A viral protein restricts *Drosophila* RNAi immunity by regulating argonaute activity and Stability. Cell Host Microbe *24*, 542–557.e9.

Nishiyama, T., Yamamoto, H., Shibuya, N., Hatakeyama, Y., Hachimori, A., Uchiumi, T., and Nakashima, N. (2003). Structural elements in the internal ribosome entry site of Plautia stali intestine virus responsible for binding with ribosomes. Nucleic Acids Res. *31*, 2434–2442.

Oerke, E. (2006). Crop losses to pests. J. Agric. Sci. *144*, 31–43.

Osborne, S.E., Leong, Y.S., O'Neill, S.L., and Johnson, K.N. (2009). Variation in antiviral protection mediated by different Wolbachia strains in *Drosophila* simulans. PLOS Pathog. *5*, e1000656. https://doi.org/10.1371/journal.ppat.1000656

Paul, A.V., and Wimmer, E. (2015). Initiation of protein-primed picornavirus RNA synthesis. Virus Res. *206*, 12–26. https://doi.org/10.1016/j.virusres.2014.12.028

Pestova, T.V., and Hellen, C.U. (2003). Translation elongation after assembly of ribosomes on the cricket paralysis virus internal ribosomal entry site without initiation factors or initiator tRNA. Genes Dev. *17*, 181–186.

Petrov, A., Grosely, R., Chen, J., O'Leary, S.E., and Puglisi, J.D. (2016). Multiple parallel pathways of translation initiation on the CrPV IRES. Mol. Cell *62*, 92–103. https://doi.org/10.1016/j.molcel.2016.03.020

Pfingsten, J.S., Costantino, D.A., and Kieft, J.S. (2006). Structural basis for ribosome recruitment and manipulation by a viral IRES RNA. Science *314*, 1450–1454.

Pfingsten, J.S., Costantino, D.A., and Kieft, J.S. (2007). Conservation and diversity among the three-dimensional folds of the Dicistroviridae intergenic region IRESes. J. Mol. Biol. *370*, 856–869.

Pimentel, D.J.E. (2005). Environmental and economic costs of the application of pesticides primarily in the United States. Development and Sustainability *7*, 229–252.

Pisareva, V.P., Pisarev, A.V., and Fernández, I.S. (2018). Dual tRNA mimicry in the cricket paralysis virus IRES uncovers an unexpected similarity with the hepatitis C virus IRES. Elife *7*, e34062. https://doi.org/10.7554/eLife.34062

Plus, N., Croizier, G., Jousset, F.X., and David, J. (1975). Picornaviruses of laboratory and wild *Drosophila* melanogaster: geographical distribution and serotypic composition. Ann. Microbiol. *126*, 107–117.

Plus, N., Croizier, G., Reinganum, C., and Scott, P.D. (1978). Cricket paralysis virus and *drosophila* C virus: serological analysis and comparison of capsid polypeptides and host range. J. Invertebr. Pathol. *31*, 296–302.

Poirier, E.Z., Goic, B., Tomé-Poderti, L., Frangeul, L., Boussier, J., Gausson, V., Blanc, H., Vallet, T., Loyd, H., Levi, L.I., *et al.* (2018). Dicer-2-dependent generation of viral DNA from defective genomes of RNA viruses modulates antiviral immunity in insects. Cell Host Microbe *23*, 353–365.e8.

Popp, J., Peto, K., and Nagy, J. (2013). Pesticide productivity and food security. A review. Agron. Sustain. Dev. *33*, 243.

Ramakrishnaiah, V., Thumann, C., Fofana, I., Habersetzer, F., Pan, Q., de Ruiter, P.E., Willemsen, R., Demmers, J.A., Stalin Raj, V., Jenster, G., *et al.* (2013). Exosome-mediated transmission of hepatitis C virus between human hepatoma Huh7.5 cells. Proc. Natl. Acad. Sci. U.S.A. *110*, 13109–13113. https://doi.org/10.1073/pnas.1221899110

Redak, R.A., Purcell, A.H., Lopes, J.R., Blua, M.J., Mizell, R.F., and Andersen, P.C. (2004). The biology of xylem fluid-feeding insect vectors of Xylella fastidiosa and their relation to disease epidemiology. Annu. Rev. Entomol. *49*, 243–270. https://doi.org/10.1146/annurev.ento.49.061802.123403

Reineke, L.C., and Lloyd, R.E. (2015). The stress granule protein G3BP1 recruits protein kinase R to promote multiple innate immune antiviral responses. J. Virol. *89*, 2575–2589. https://doi.org/10.1128/JVI.02791-14

Reinganum, C. (1975). The isolation of cricket paralysis virus from the emperor gum moth, Antheraea eucalypti Scott, and its infectivity towards a range of insect species. Intervirology *5*, 97–102. https://doi.org/10.1159/000149886

Reinganum, C., O'Loughlin, G.T., and Hogan, T.W. (1970). A nonoccluded virus of the field crickets *Teleogryllus oceanicus* and *T. commodus* (Orthoptera: Gryllidae). J. Invertebr. Pathol. *16*, 214–220.

Ren, Q., Wang, Q.S., Firth, A.E., Chan, M.M., Gouw, J.W., Guarna, M.M., Foster, L.J., Atkins, J.F., and Jan, E. (2012). Alternative reading frame selection mediated by a tRNA-like domain of an internal ribosome entry site. Proc. Natl. Acad. Sci. U.S.A. *109*, E630–9. https://doi.org/10.1073/pnas.1111303109

Ren, Q., Au, H.H., Wang, Q.S., Lee, S., and Jan, E. (2014). Structural determinants of an internal ribosome entry site that direct translational reading frame selection. Nucleic Acids Res. *42*, 9366–9382. https://doi.org/10.1093/nar/gku622

Robalino, J., Browdy, C.L., Prior, S., Metz, A., Parnell, P., Gross, P., and Warr, G. (2004). Induction of antiviral immunity by double-stranded RNA in a marine invertebrate. J. Virol. *78*, 10442–10448. https://doi.org/10.1128/JVI.78.19.10442-10448.2004

Robalino, J., Bartlett, T.C., Chapman, R.W., Gross, P.S., Browdy, C.L., and Warr, G.W. (2007). Double-stranded RNA and antiviral immunity in marine shrimp: inducible host mechanisms and evidence for the evolution of viral counter-responses. Dev. Comp. Immunol. *31*, 539–547.

Roberts, L.O., and Groppelli, E. (2009). An atypical IRES within the 5' UTR of a dicistrovirus genome. Virus Res. *139*, 157–165. https://doi.org/10.1016/j.virusres.2008.07.017

Ruehle, M.D., Zhang, H., Sheridan, R.M., Mitra, S., Chen, Y., Gonzalez, R.L., Cooperman, B.S., and Kieft, J.S. (2015). A dynamic RNA loop in an IRES affects multiple steps of elongation factor-mediated translation initiation. Elife *4*, e08146. https://doi.org/10.7554/eLife.08146

Sabath, N., Price, N., and Graur, D. (2009). A potentially novel overlapping gene in the genomes of Israeli acute paralysis virus and its relatives. Virol. J. *6*, 144. https://doi.org/10.1186/1743-422X-6-144

Saleh, M.C., van Rij, R.P., Hekele, A., Gillis, A., Foley, E., O'Farrell, P.H., and Andino, R. (2006). The endocytic pathway mediates cell entry of dsRNA to induce RNAi silencing. Nat. Cell Biol. *8*, 793–802.

Saleh, M.C., Tassetto, M., van Rij, R.P., Goic, B., Gausson, V., Berry, B., Jacquier, C., Antoniewski, C., and Andino, R. (2009). Antiviral immunity in *Drosophila* requires systemic RNA interference spread. Nature *458*, 346–350. https://doi.org/10.1038/nature07712

Sánchez-Eugenia, R., Goikolea, J., Gil-Cartón, D., Sánchez-Magraner, L., and Guérin, D.M. (2015). Triatoma virus recombinant VP4 protein induces membrane permeability through dynamic pores. J. Virol. *89*, 4645–4654. https://doi.org/10.1128/JVI.00011-15

Sansone, C.L., Cohen, J., Yasunaga, A., Xu, J., Osborn, G., Subramanian, H., Gold, B., Buchon, N., and Cherry, S. (2015). Microbiota-dependent priming of antiviral intestinal immunity in *Drosophila*. Cell Host Microbe *18*, 571–581. https://doi.org/10.1016/j.chom.2015.10.010

Sasaki, J., and Nakashima, N. (1999). Translation initiation at the CUU codon is mediated by the internal ribosome entry site of an insect picorna-like virus in vitro. J. Virol. *73*, 1219–1226.

Sasaki, J., and Nakashima, N. (2000). Methionine-independent initiation of translation in the capsid protein of an insect RNA virus. Proc. Natl. Acad. Sci. U.S.A. *97*, 1512–1515. https://doi.org/10.1073/pnas.010426997

Schüler, M., Connell, S.R., Lescoute, A., Giesebrecht, J., Dabrowski, M., Schroeer, B., Mielke, T., Penczek, P.A., Westhof, E., and Spahn, C.M. (2006). Structure of the ribosome-bound cricket paralysis virus IRES RNA. Nat. Struct. Mol. Biol. *13*, 1092–1096.

Serbus, L.R., and Sullivan, W. (2007). A cellular basis for Wolbachia recruitment to the host germline. PLOS Pathog. *3*, e190.

Shi, M., Lin, X.D., Tian, J.H., Chen, L.J., Chen, X., Li, C.X., Qin, X.C., Li, J., Cao, J.P., Eden, J.S., *et al.* (2016). Redefining the invertebrate RNA virosphere. Nature [Epub ahead of print]. https://doi.org/10.1038/nature20167

Snijder, J., Uetrecht, C., Rose, R.J., Sanchez-Eugenia, R., Marti, G.A., Agirre, J., Guérin, D.M., Wuite, G.J., Heck, A.J., and Roos, W.H. (2013). Probing the biophysical interplay between a viral genome and its capsid. Nat. Chem. *5*, 502–509. https://doi.org/10.1038/nchem.1627

Spahn, C.M., Jan, E., Mulder, A., Grassucci, R.A., Sarnow, P., and Frank, J. (2004). Cryo-EM visualization of a viral internal ribosome entry site bound to human ribosomes: the IRES functions as an RNA-based translation factor. Cell *118*, 465–475. https://doi.org/10.1016/j.cell.2004.08.001

Spurny, R., Přidal, A., Pálková, L., Kiem, H.K., de Miranda, J.R., and Plevka, P. (2017). Virion structure of black queen cell virus, a common honeybee pathogen. J. Virol. *91*, e02100–16.

Squires, G., Pous, J., Agirre, J., Rozas-Dennis, G.S., Costabel, M.D., Marti, G.A., Navaza, J., Bressanelli, S., Guerin, D.M., and Rey, F.A. (2013). Structure of the Triatoma virus capsid. Acta Crystallogr. D Biol. Crystallogr. *69*, 1026–1037.

Talide, L., Imler, J.L., and Meignin, C. (2019). Sensing viral infections in insects: pathways looking for receptors. In Insect Molecular Virology: Advances and Emerging Trends, B.C. Bonning, ed. (Caister Academic Press, Norfolk, UK).

Tassetto, M., Kunitomi, M., and Andino, R. (2017). Circulating immune cells mediate a systemic RNAi-based adaptive antiviral response in *Drosophila*. Cell *169*, 314–325.e13.

Tate, J., Liljas, L., Scotti, P., Christian, P., Lin, T., and Johnson, J.E. (1999). The crystal structure of cricket paralysis virus: the first view of a new virus family. Nat. Struct. Biol. *6*, 765–774. https://doi.org/10.1038/11543

Teixeira, L., Ferreira, A., and Ashburner, M. (2008). The bacterial symbiont Wolbachia induces resistance to RNA viral infections in *Drosophila* melanogaster. PLOS Biol. *6*, e2. https://doi.org/10.1371/journal.pbio.1000002

Thompson, S.R., Gulyas, K.D., and Sarnow, P. (2001). Internal initiation in Saccharomyces cerevisiae mediated by an initiator tRNA/eIF2-independent internal ribosome entry site element. Proc. Natl. Acad. Sci. U.S.A. *98*, 12972–12977. https://doi.org/10.1073/pnas.241286698

Toriyama, S., Guy, P.L., Fuji, S., and Takahashi, M. (1992). Characterization of a new picorna-like virus, himetobi P virus, in planthoppers. J. Gen. Virol. *73*, 1021–1023. https://doi.org/10.1099/0022-1317-73-4-1021

Tufts, D.M., Hunter, W.B., and Bextine, B. (2014). Solenopsis invicta virus (Sinv-1) infection and insecticide interactions in the red imported fire ant (Hymenoptera: Formicidae). Fla. Entomol. *97*, 1251–1254.

Valles, S.M. (2012). Positive-strand RNA viruses infecting the red imported fire ant, *Solenopsis invicta*. Psyche *2012*, 14. https://doi.org/10.1155/2012/821591

Valles, S.M., and Hashimoto, Y. (2009). Isolation and characterization of Solenopsis invicta virus 3, a new positive-strand RNA virus infecting the red imported fire ant, Solenopsis invicta. Virology *388*, 354–361. https://doi.org/10.1016/j.virol.2009.03.028

Valles, S.M., Chen, Y., Firth, A.E., Guérin, D.M.A., Hashimoto, Y., Herrero, S., de Miranda, J.R., and Ryabov, E. (2017). ICTV virus taxonomy profile: Dicistroviridae. J. Gen. Virol. *98*, 355–356.

van Rij, R.P., Saleh, M.C., Berry, B., Foo, C., Houk, A., Antoniewski, C., and Andino, R. (2006). The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in *Drosophila* melanogaster. Genes Dev. *20*, 2985–2995.

Wamonje, F.O., Michuki, G.N., Braidwood, L.A., Njuguna, J.N., Musembi Mutuku, J., Djikeng, A., Harvey, J.J.W., and Carr, J.P. (2017). Viral metagenomics of aphids present in bean and maize plots on mixed-use farms in Kenya reveals the presence of three dicistroviruses including a novel Big Sioux River virus-like dicistrovirus. Virol. J. *14*, 188. https://doi.org/10.1186/s12985-017-0854-x

Wang, Q.S., Au, H.H., and Jan, E. (2013). Methods for studying IRES-mediated translation of positive-strand RNA viruses. Methods *59*, 167–179. https://doi.org/10.1016/j.ymeth.2012.09.004

Wang, X.H., Aliyari, R., Li, W.X., Li, H.W., Kim, K., Carthew, R., Atkinson, P., and Ding, S.W. (2006). RNA interference directs innate immunity against viruses in adult *Drosophila*. Science *312*, 452–454.

Watanabe, M., Iwakawa, H.O., Tadakuma, H., and Tomari, Y. (2017). Biochemical and single-molecule analyses of the RNA silencing suppressing activity of CrPV-1A. Nucleic Acids Res. *45*, 10837–10844. https://doi.org/10.1093/nar/gkx748

White, J.P., Cardenas, A.M., Marissen, W.E., and Lloyd, R.E. (2007). Inhibition of cytoplasmic mRNA stress granule formation by a viral proteinase. Cell Host Microbe *2*, 295–305.

Williams, G.R., Tarpy, D.R., vanEngelsdorp, D., Chauzat, M.P., Cox-Foster, D.L., Delaplane, K.S., Neumann, P., Pettis, J.S., Rogers, R.E., and Shutler, D. (2010). Colony Collapse Disorder in context. Bioessays *32*, 845–846. https://doi.org/10.1002/bies.201000075

Williamson, C., Rybicki, E.P., Kasdorf, G.G.F., and Von Wechmar, M.B. (1988). Characterization of a new Picorna-like virus isolated from aphids. J. Gen. Virol. *69*, 787–795.

Wilson, J.E., Pestova, T.V., Hellen, C.U., and Sarnow, P. (2000a). Initiation of protein synthesis from the A site of the ribosome. Cell *102*, 511–520.

Wilson, J.E., Powell, M.J., Hoover, S.E., and Sarnow, P. (2000b). Naturally occurring dicistronic cricket paralysis virus RNA is regulated by two internal ribosome entry sites. Mol. Cell. Biol. *20*, 4990–4999.

Wilson, R.C., and Doudna, J.A. (2013). Molecular mechanisms of RNA interference. Annu. Rev. Biophys. *42*, 217–239. https://doi.org/10.1146/annurev-biophys-083012-130404

Wong, Z.S., Brownlie, J.C., and Johnson, K.N. (2015). Oxidative stress correlates with Wolbachia-mediated antiviral protection in Wolbachia-*Drosophila* associations. Appl. Environ. Microbiol. *81*, 3001–3005. https://doi.org/10.1128/AEM.03847-14

Wong, Z.S., Brownlie, J.C., and Johnson, K.N. (2016). Impact of ERK activation on fly survival and Wolbachia-mediated protection during virus infection. J. Gen. Virol. *97*, 1446–1452. https://doi.org/10.1099/jgv.0.000456

Woolaway, K.E., Lazaridis, K., Belsham, G.J., Carter, M.J., and Roberts, L.O. (2001). The 5' untranslated region of Rhopalosiphum padi virus contains an internal ribosome entry site which functions efficiently in mammalian, plant, and insect translation systems. J. Virol. *75*, 10244–10249. https://doi.org/10.1128/JVI.75.21.10244-10249.2001

Wu, Q., Luo, Y., Lu, R., Lau, N., Lai, E.C., Li, W.X., and Ding, S.W. (2010). Virus discovery by deep sequencing and assembly of virus-derived small silencing RNAs. Proc. Natl. Acad. Sci. U.S.A. *107*, 1606–1611. https://doi.org/10.1073/pnas.0911353107

Wu, X., He, W.T., Tian, S., Meng, D., Li, Y., Chen, W., Li, L., Tian, L., Zhong, C.Q., Han, F., *et al.* (2014). pelo is required for high efficiency viral replication. PLOS Pathog. *10*, e1004034. https://doi.org/10.1371/journal.ppat.1004034

Xu, J., Hopkins, K., Sabin, L., Yasunaga, A., Subramanian, H., Lamborn, I., Gordesky-Gold, B., and Cherry, S. (2013). ERK signaling couples nutrient status to antiviral defense in the insect gut. Proc. Natl. Acad. Sci. U.S.A. *110*, 15025–15030. https://doi.org/10.1073/pnas.1303193110

Yamamoto, H., Nakashima, N., Ikeda, Y., and Uchiumi, T. (2007). Binding mode of the first aminoacyl-tRNA in translation initiation mediated by Plautia stali intestine virus internal ribosome entry site. J. Biol. Chem. *282*, 7770–7776.

Yang, S., Yu, J., Fan, Z., Gong, S.T., Tang, H., and Pan, L. (2018). Bub1 facilitates virus entry through endocytosis in a model of *Drosophila* pathogenesis. J. Virol. *92*, e00254–18.

Zeidler, M.P., and Bausek, N. (2013). The *Drosophila* JAK-STAT pathway. JAKSTAT *2*, e25353. https://doi.org/10.4161/jkst.25353

Zhang, H., Ng, M.Y., Chen, Y., and Cooperman, B.S. (2016). Kinetics of initiating polypeptide elongation in an IRES-dependent system. Elife *5*, e13429. https://doi.org/10.7554/eLife.13429

Zhang, Q., Zhang, L., Gao, X., Qi, S., Chang, Z., and Wu, Q. (2015). DIP1 plays an antiviral role against DCV infection in *Drosophila* melanogaster. Biochem. Biophys. Res. Commun. *460*, 222–226. https://doi.org/10.1016/j.bbrc.2015.03.013

Zhu, J., Korostelev, A., Costantino, D.A., Donohue, J.P., Noller, H.F., and Kieft, J.S. (2011). Crystal structures of complexes containing domains from two viral internal ribosome entry site (IRES) RNAs bound to the 70S ribosome. Proc. Natl. Acad. Sci. U.S.A. *108*, 1839–1844. https://doi.org/10.1073/pnas.1018582108