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RNA virus EVEs in insect genomes

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Insects are infected by a diverse set of RNA viruses that are more broadly distinguished by their ability to infect single or multiple host species. During replication into the host cell, partial or complete double strand DNA derived from the viral genome may be integrated into their host genomes giving origin to endogenous viral elements (EVEs). EVEs from RNA viruses have been identified in a variety of insect genomes showing different evolutionary trajectories: from highly degraded viral genomic remains to partial and complete viral coding regions. Limited functional knowledge exists about RNA EVEs impact on hosts and circulating viruses, but exciting results are emerging showing a complex arms race interplay that influences the evolutionary trajectory of these interacting entities.

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Genomes in flux – exchange of genetic material between host and viral genomes

Viruses emerged independently multiple times during the history of life, they are likely descendants of one the first nucleic acid replicators and abundant evidence also shows that many lineages emerged as defective or hybrid versions of prokaryotic and eukaryotic cells [1,2]. These host-dependent replicating entities show several characteristics of rapid adaptation due to features such as high mutation rate and a particular ability to exchange pieces of its genome with other viruses and their host cell genomes [3–5]. A continuum arms race between viruses and hosts have unleashed a number of biological innovations such as the emergence of CRISPR-Cas molecular defense system in prokaryotes, small regulatory RNA in eukaryotes and several co-option events of viral genes that were repurposed and currently perform critical function in the host biology such as the formation and function of mammals placenta [6–9]. But only recently, with the availability of many prokaryotic and eukaryotic genomes, researchers truly realized the contribution and impact of viral-derived sequences integrated into the host genome on the arms race dynamics and host biology [3].

How EVEs emerge from viruses lacking key molecules for dsDNA production and integration?

Except for certain viruses, such as retroviruses and bacteriophages that code for reverse transcriptases (RT) and integrases (INT), most viruses are not expected to be integrated in the genome of their hosts since they lack those enzymes [10]. Yet, several examples of RNA viruses EVEs, covering all known genome structure and replication strategies, can be found in eukaryotic genomes including insect genomes [3,11]. These elements are commonly known as non-retroviral integrated RNA virus sequences (NIRVS) or simply endogenous viral elements (EVEs).

There are a couple of evidences suggesting that transposable elements (TEs), and more specifically retrotransposons, are involved into reverse transcription and integration of viral genomic fragments: (I) – viral RNA genomes are known to be reverse transcribed to viral double strand DNA (vDNA) by reverse transcriptases (RT) molecules in insects and the sole major source of these molecules are those coding by the abundant retrotransposons which inhabit insect genomes [12–14] – an important remark is that non integrated vDNA generated during reverse transcription are known to produce antiviral sRNA which has a critical role in viral tolerance of mosquitoes [12]; (II) – Both retrotransposons and non-retroviral RNA viruses replication cycle occurs in the cytoplasm, that is, time and subcellular localization opportunity exists for reverse transcription of RNA genomes into vDNA by retrotransposons RTs; (III) – circular DNA molecules (also known as episomes) containing adjacent retrotransposons and vDNA fragments have been found in mosquitoes cells [15••]; and (IV) – EVEs are majoritarily found integrated into TE and particularly retrotransposon rich regions of the host genome usually flanked by retrotransposons LTRs [15••,16•,17•]. Therefore, the most likely hypothesis to explain the mechanism of viral integration of RNA viruses genomes is that RNA genomic sequences are loaded into replication complexes of retrotransposons, are reverse transcribed to viral double-strand DNA (vDNA) giving origin to retrotransposon/virus episomal recombinants that are then integrated into

the host genomes by retrotransposons integrases or non-homologous recombination [15••,18•].

Retrotransposon/virus DNA hybrids are likely generated by non-homologous recombination during reverse transcription by a copy-choice mechanism [19••]. Evidence of episomal retrotransposon/virus DNA hybrids and several NIRVS integration sites associated with long-terminal repeats (LTRs) of retrotransposons found in mosquitoes and fruit fly genomes support that template switching during retrotransposon/virus replication is the major mechanism of episomal retrotransposon/vDNA hybrids formation [15••,20]. However, only limited indirect evidence (enrichment of NIRVS in TE rich loci) is available for other insect species so far (Figure 1).

Which factors may impact viral genomic fragments integration and long term persistence in insect genomes?

Viral genomic integration into germline cells genomes is key for NIRVS inheritance to the next host generation and hence different virus features may influence the integration likelihood at germline cells such as tissue tropism and subcellular site of viral replication. Viruses that are vertically transmitted are known to replicate and infect host germinative cells at a higher rate than horizontally transmitted viruses and hence have a higher likelihood of integration into these cells [10]. Another viral feature that may impact its integration likelihood in the host genome is the establishment of persistent or transient infection of the host: Viruses that persistently infect host cells have a broader window of opportunity to generate and integrate vDNA than viruses that undergo a transient acute viral infection [10]. Moreover, transient acute infections are generally more detrimental to the host leading to decreased host fitness and lower chance of integrated vDNAs to succeed in the next host generation [11].

Reverse transcription and integration are two important steps in the generation of vDNA and its endogenization into the host genome. Therefore, it is reasonable to speculate that host retrotransposon activity, the sole RTs and INTs molecules source from the insect genomes, will impact vDNA formation and integration rate, although more detailed molecular mechanism of virus genome and retrotransposons interaction will be needed to tease apart the role of specific retrotransposon families/superfamilies activity on general pattern of NIRVS emergence.

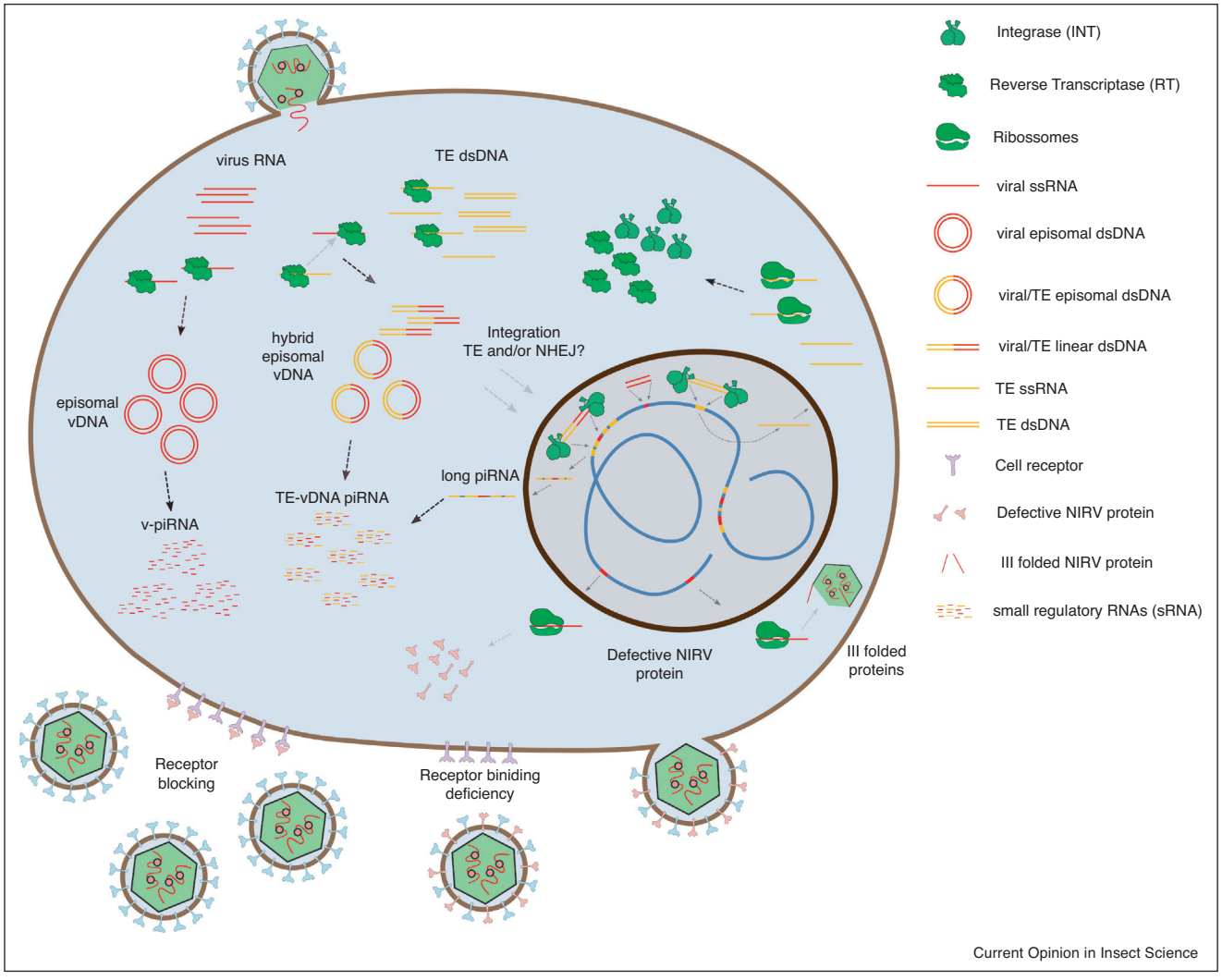
At the moment that a new NIRV loci emerges into the germline cell genome it becomes a host allele and is subject to evolutionary forces acting upon the host level as well. It means that its likelihood of fixation or loss in a given population/species is dependent on their impact on host fitness. First, the host individual should be fit to reproduce and hence generate descendants that will successfully mate and spread the NIRV loci into the population. That is, vDNA integrations with detrimental effect to the host may be lost in a single host generation and only slight deleterious, neutral or advantageous insertions may spread to the host population. Species population size and NIRV allele frequency may impact the short term allele fluctuations. Small populations are subject to a larger impact of genetic drift, and non fixed NIRVs loci may be lost in a few generations or slightly deleterious NIRV alleles may increase in frequency just by chance. On the other hand, large populations are subject to strong purifying selection eliminating deleterious insertion rapidly and/or increasing rapidly the frequency of beneficial NIRV alleles. Therefore, the host evolutionary history at short and long time scales should be also taken into account to fully understand NIRVs loci evolutionary trajectory and current evolutionary pressures acting upon them.

What is known from insects genomes

The first NIRV found in insect genomes were Flavivirus-related sequences identified in mosquito cell lines, lab breed and natural populations of *Aedes aegypti* and *Aedes albopictus* [21]. Since then, a wide breadth of viral families have been found endogenized into insect genomes including families of linear positive sense single strand RNA genomes such as Flaviviridae and Virgaviridae; segmented linear single strand positive sense RNA genome such as Benyvirus; linear negative sense single strand RNA genomes such as: *Rhabdoviridae*, *Chuviridae*, *Xinmoviridae* and *Peribunyaviridae*; segmented negative sense single strand RNA genomes as Orthomyxoviridae and Phenuiviridae; and segmented double strand RNA genomes such as Reoviridae [11,18•,22–24].

Two families of — ssRNA viruses, Rhabdoviridae and Chuviridae, appear to integrate more frequently in insect genomes, which may reflect the high abundance and diversity of these viruses as well as its diverse ecology and insect host range [11]. However, we still have a limited idea about the complete virome of insects hindering more broad and unbiased analysis of virome diversity/abundance and endogenization trends. Most unbiased metatranscriptomic studies conducted so far are focused on insects of medical importance and on few individuals/populations of a given species [11,25–29]. In fact, the few metatranscriptomic studies including a wide range of Insect taxa were able to characterize hundreds of new viral complete genomes and also provided the most extensive characterization of NIRVS in diverse insect taxa so far [30••,31••]. NIRVS have been found in at least 7 orders — Diptera, Hymenoptera, Hemiptera, Coleoptera, Lepidoptera, Blattodea (Dictyoptera) and Thysanoptera among the 28–30 insect orders currently recognized (https://www.royensoc.co.uk/insect-classification),

Figure 1



Main concepts covered in this review regarding NIRVS emergence mechanisms and impact on virus-host coevolution. Red rectangles are single strand RNA derived from viral genomes and NIRVS, Yellow rectangles are single strand RNA from transposable elements, parallel rectangles and circular molecules are double strand DNA derived from viral reverse transcription (red) and viral/retrotransposon non-homologous recombinants. Linear yellow, blue and red rectangle is a long piRNA precursor produced from piRNA clusters bearing TEs (yellow) and NIRVS (red). Small yellow and red rectangles are piRNAs derived from vDNA, virus/retrotransposon circular DNA hybrids and long piRNA precursors. NHEJ – Non-Homologous End Joining. Black arrows denote mechanistic molecular steps of NIRVS emergence and functional impact with direct evidence available while light grey arrows denote hypothesis with only indirect evidence available or steps that remains to be evaluated.

but large differences exists on the diversity of species from each group studied [24,32••,33,34,35•]. The large majority NIRVS found in insect genomes so far were described in mosquitos from the *Aedes* genus which may reflect the large diversity of RNA viruses that infect this particular mosquito genus [17•]. Therefore, the true contribution of RNA viruses to the emergence of NIRVS in insect species remains to be assessed systematically in a large diversity of insect genomes. Many more NIRVS are expected to be found as we expand our knowledge of the insect–virus genomic diversity.

NIRVS impact on insect–virus coevolution

What evolutionary pressure NIRVS are subject to after its emergence?

Exogenous circulating RNA viruses evolve at a high substitution rate compared to their host genome, which is mainly propelled by their error prone RNA polymerases and several rounds of selection and counter selection during virus–host arms race [22]. However, once a viral genomic fragment is integrated into the host genome, it is then subject to the host and virus selection pressure leading to different evolutionary scenarios depending

of the host fitness impact: (I) – the integration event occurred in a silent genomic loci then such NIRV is expected to evolve neutrally and accumulate mutations until it could no longer be recognized or lost; (II) – NIRVS are integrated in transcriptionally active *loci* contributing to the biogenesis of small RNAs (sRNA), which in turn modulate the replication of the cognate viruses. Once the cognate virus infection has a deleterious fitness impact on the host then a sRNA producing NIRV *loci* will evolve under intermittent purifying and diversifying selection at the most critical sRNA sites for cognate viral recognition as long as the exogenous RNA viruses continue to infect the host; (III) – NIRVS are transcriptionally and translationally active generating protein sequences that can interfere with the cognate viral replication/infection, these would also evolve under intermittent purifying and diversifying selection which will be more evident at the amino acid level; and (IV) – NIRVS derived proteins are repurposed to a new host function and the selection pressure regime will be dictated by the importance of such protein to the host fitness. A detailed review of evolutionary dynamics of EVEs can be found in Aswad and Katzourakis *et al.* [36].

Mounting evidence in insects, but mostly on mosquitoes, shows that most NIRVS are transcriptionally active [17•,37,38•] and produce small interfering RNAs (sRNAs) [16•,17•,32••,34,39–41]. However, the large majority of these NIRVS are substantially divergent at the nucleotide level of any possible cognate exogenous virus, casting doubts about the role of these elements in the regulation of cognate viruses replication through sequence dependent sRNA pathways [32••]. The first 'proof of concept' study showing experimental evidence of NIRVS regulatory role on exogenous virus replication through piRNA (PIWI)-interacting RNAs (piRNAs) was based on the engineering of a naturally occurring RNA NIRV in a RNA virus genome. Tasseto *et al.* showed that the expression of v-piRNA (viral derived piRNA) from NIRV loci controls the infection of the engineered virus [15••], but experimental evidence in a natural insect–virus setting was still lacking. Only two cases of NIRVS that share a high similarity to cognate viruses are currently known. The sigma related NIRVS found in *Drosophila* species, which are persistently infected by the sigma virus [20,42,43], and the Cell Fusion Agent Virus (CFAV) NIRV found integrated into *A. aegypti* populations naturally infected by this virus [18•,19••,44]. From the last example emerged one of the most compelling evidences of the antiviral role of NIRVS derived sRNAs in the modulation of cognate virus replication. Suzuki *et al.* were able to tease apart the contribution of v-piRNA derived from episomal or NIRV and showed that when generating an engineered *A. aegypti* NIRV-free, the bulk v-piRNA production was substantially reduced and the cognate virus replicated at higher levels [19••].

On the other hand, no study investigated the impact of insect NIRVS at the protein functional level regarding potential antiviral activity or repurpotioning to other functions at the host level, although there is compelling indirect evidence from genomic studies. Recent findings have reported NIRVS open reading frames fused or not to host genes that are actively sense transcribed [22,35•,37,45]. Moreover, antiviral mechanisms of partial, defective or ill folded viral proteins are also known for vertebrates [36,46,47]. An interesting model to test some of these hypotheses about the role of protein derived from NIRVS come from mosquito genomic studies. Several NIRVS from Chuviruses, segmented and non-segmented single stranded negative sense viruses, have been found in mosquitoes (mainly in *A. aegypti and A. albopictus* species) with a higher abundance of potentially coding loci for complete or partial glycoproteins [16•,48•]. Many highly similar glycoproteins with coding capacity were found into potentially functional BEL-Pao retrotransposons (parental BEL-Pao lineages previously lacking glycoproteins) suggesting that this glycoprotein is currently functioning as an envelope gene of a new retrovirus called 'Anakin'. Moreover, several solo complete or partial glycoprotein with high sequence similarity and similar predicted 3D structure to the glycoprotein found inside BEL-Pao retroelements can also be found scattered in the mosquito genome. Such potentially coding solo glycoproteins may be translated and act as an antiviral mechanism against this new retrovirus and/or exogenous Chuviruses by receptor competition [48•]. However, experimental evidences of antiviral mechanisms or co-option of NIRVS to new host functions at the proteome level in insects are still not available and remains an interesting field of research.

Conclusions

The complex interactions of RNA viruses and insects leave heritable changes in the host genomes in the form of partial or complete viral genomes that may substantially impact the virus–insect coevolution arms race. NIRV-derived regulatory piRNA modulates cognate virus replication having an impact on host tolerance to virus detrimental effect and likely on vectorial competence. Nonetheless, NIRVS impact on host–virus biology remains to be more extensively demonstrated in natural insect–virus settings. NIRVS emergence is a dynamic and frequent phenomenon and our current knowledge only sheds light on the tip of the iceberg of NIRVS in insects.

Conflict of interest statement

Nothing declared.

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