Title

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Journal

Cell Host & Microbe, 23(3)

ISSN

1931-3128

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Publication Date

2018-03-01

DOI

10.1016/j.chom.2018.02.010

Peer reviewed

ROS production. It is expected that the initial burst of ROS will depend on the NADPH pool present in the cells and that only long-term ROS production will require the metabolic switch.

The work by Lee et al. focuses on one arm of the antimicrobial response, ROS production. However, it is likely that the TRAF3/WTS pathway will also interfere with other immune signaling cascades in the gut that share components or metabolic hubs with this pathway. The most obvious candidates are the Toll and immune deficiency (IMD) NF-κB cascades that regulate antimicrobial peptide (AMP) production in response to bacterial peptidoglycan, a ligand previously shown to stimulate lipolysis (Chi et al., 2014). The IMD pathway shows strong similarities with the mammalian TNFα pathway in which TRAFs play an important role, suggesting that TRAF3 levels could also affect IMD pathway activity (Ganesan et al., 2011). In addition, it has been shown that IMD activation antagonizes S6K (S6 kinase) and AKT activation, which results in downregulation of anabolism (Clark et al., 2013). This study shows that TRAF3-dependent ROS production is also mediated by AKT inhibition. Finally, activation of the Toll receptor by Gram-positive bacteria suppresses transcription of the Iκ-B kinase through the Hippo-WTS pathway in another immune-competent tissue, the fat body (Liu et al., 2016). More work in this exciting area of research will no doubt provide us with a more integrated view of the functional links that take place between the immune and metabolic pathways to ensure optimal and durable protection for the host.

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Templating Antiviral RNAi in Insects

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https://doi.org/10.1016/j.chom.2018.02.010

Virus-specific small interfering RNAs (siRNAs) are a central component of antiviral responses in insects. In this issue of *Cell Host & Microbe*, Poirier et al. (2018) demonstrate that virus-infected flies and mosquitoes produce virus-derived extrachromosomal circular DNAs that serve as a template for the biogenesis of antiviral siRNAs.

Antiviral RNA interference (RNAi) clears virus infection in plants and animals by a highly conserved genetic pathway (Ding, 2010). The antiviral RNAi pathway begins with Dicer-mediated processing of virus-specific double-strand RNA (dsRNA) into small interfering RNAs (siRNAs). Subsequently, these virus-derived siRNAs (vsiRNAs) guide specific viral RNA clearance by an Argonaute protein in RNA-induced silencing complex (Figure 1). Effective antiviral RNAi in plants and nematodes depends on the amplification of the vsiRNAs by a related family of host RNA-dependent RNA polymerases (RdRPs) following the biogenesis of the primary vsiRNAs processed from viral dsRNA replicative intermediates (Ding, 2010). However, it has been unclear why the total vsiRNAs are as abundant in fruit flies as in nematodes, which do not encode an

RdRP homolog. In a recent study, Poirier et al. (2018) demonstrate the production of vsiRNAs in the infected fruit flies templated by the viral extrachromosomal circular DNA (eccDNA), providing a novel pathway for the biogenesis of the vsiRNAs.

Previous work has detected DNA fragments that are reverse transcribed from RNA viruses and embedded in retrotransposon sequences in the infected fruit flies (Goic et al., 2013; Tassetto et al., 2017). Viral DNA production is part of the insect antiviral RNAi response since inhibiting reverse transcription with drug AZT enhances virus susceptibility and reduces the biogenesis of vsiRNAs (Goic et al., 2013; Tassetto et al., 2017). Since most retrotransposons produce circular DNA after reverse transcription, Poirier et al. (2018) searched for the presence of circular viral DNA in fruit fly S2 cells and adults after infection with Flock house virus (FHV), which contains a bipartite positive-strand RNA genome. Using an ATP-dependent DNase to remove linear DNA, Poirier et al. (2018) showed that FHV-derived eccDNA molecules accumulate to levels readily detectable by PCR both *in vitro* and *in vivo*. Further unbiased deep sequencing of the eccDNA molecules enriched from infected S2 cells confirmed that FHV-derived eccDNA molecules comprise a heterogeneous population of chimeric, partial, and truncated viral genomic sequences similar to those characterized previously (Goic et al., 2013).

Notably, Poirier et al. (2018) showed that injection of the total eccDNA isolated from FHV-infected S2 cells into naive fruit flies triggers the production of a population of vsiRNAs similar to those made by Dicer-2 in virus-infected cells (Ding, 2010). These eccDNA-induced vsiRNAs are predominantly 21 nt in length, have 5′ monophosphate ends, and are divided approximately equally into positive and negative strands. Interestingly, these eccDNA-induced vsiRNAs are nearly uniformly mapped to all regions of both the viral positive- and the viral negative-strand genomic RNAs, indicating transcription and Dicer-2 processing of FHV genome-wide vsiRNA precursor RNAs from the viral eccDNA molecules in the injected flies. Moreover, the distribution pattern of the eccDNA-induced vsiRNAs differs from those of the vsiRNAs triggered by FHV infection either with or without the suppression of antiviral RNAi by the B2 protein, a viral suppressor of RNAi (VSR). The vsiRNAs from FHV-infected flies are mostly positive strands due to viral suppression of RNAi whereas much higher densities of the vsiRNAs target the 5′-terminal regions of the viral genomic RNAs in FHVΔB2-infected flies (Han et al., 2011). The distinct distribution pattern of the eccDNA-induced vsiRNAs suggests that they are produced in the injected flies that do not express a functional viral RdRP or VSR. Intriguingly, Poirier et al. (2018) found that in contrast to the total viral eccDNA, individually cloned, single-copy viral eccDNA molecules fail to induce the biogenesis of vsiRNAs in either S2 cells or flies. Therefore, the dsRNA precursors of the vsiRNAs may form either between sense and antisense transcripts from different single-copy eccDNA molecules or intramolecularly from transcripts of individual multiple-repeat eccDNA molecules.

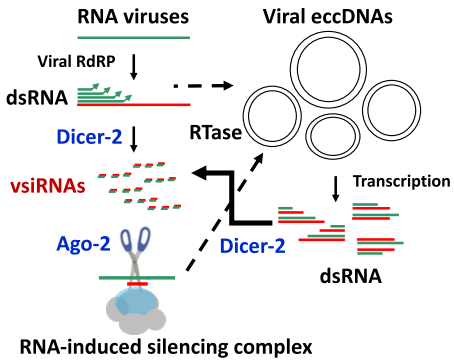

Figure 1. Two Biogenesis Pathways for Insect Virus-Derived siRNAs

Dicer-2 processes dsRNA precursors synthesized either by viral RNA replication or by transcription from viral eccDNA molecules to produce two populations of insect virus-derived siRNAs (vsiRNAs) in infected cells. RdRP, RNA-dependent RNA polymerase encoded by positive-, negative- or double-strand RNA viruses; RTase, reverse transcriptase encoded by retrotransposons; Ago-2, Argonaute-2.

Poirier et al. (2018) further provided evidence that viral eccDNA-induced vsiRNAs are functional *in vivo*. They showed that injection of the total eccDNA from FHV-infected S2 cells induces a modest, but statistically significant, increase in the survival time of the injected flies against subsequent infection with FHV. The induced protection is virus specific since the injected flies are not protected against *Drosophila* C virus (DCV), a positive-strand RNA virus unrelated to FHV. However, the increased survival of the injected flies is not accompanied by a decrease in virus titers. The eccDNA-induced, RNAi-mediated virus resistance is not as effective as expected probably because of the potent VSR encoded by FHV. Alternatively, and consistent with the antiviral function of the primary and secondary vsiRNAs in plants and nematodes (Ding, 2010), potent antiviral RNAi in insects may require the two genetically distinct populations of vsiRNAs processed by Dicer-2 from dsRNA precursors synthesized by both viral RNA replication and RNA transcription from the viral eccDNAs (Figure 1).

Poirier et al. (2018) also illustrated production of viral eccDNA in fruit flies infected with DCV and Sindbis virus (Sindbis) as well as in mosquitoes infected with chikungunya virus (CHIKV), suggesting that viral eccDNA biogenesis is a conserved feature of insect RNA virus infection. Sindbis and CHIKV are mosquito-transmitted positive-strand RNA viruses of the alphavirus genus and both are known to produce defective viral genomes (DVGs), which contain large genomic deletions and can inhibit its helper virus infection. Poirier et al. (2018) showed that both Sindbis and its DVG RNAs serve as templates for linear and circular forms of viral DNA. Further genetic studies using specific mutant flies demonstrated that the DVG-stimulated antiviral response depends on an intact antiviral RNAi response, but not on Jak-Stat/Imd innate immune signaling.

Poirier et al. (2018) further examined a panel of *dicer-2* fly mutants for the production of eccDNA and vsiRNAs. The N-terminal ATPase domain of Dicer-2 exhibits dsRNA unwinding activity and is necessary for ATP-dependent processing of long dsRNA into siRNA (Sinha et al., 2018). Interestingly, Poirier et al. (2018) identified mutations in the ATPase domain that inhibited production of eccDNA more effectively than production of vsiRNAs, suggesting a dual role for Dicer-2 in the production of both eccDNA and vsiRNAs.

In conclusion, this body of work provides an example for the production and function of a distinct class of eukaryotic

eccDNAs, which have been discovered in a wide range of other species (Paulsen et al., 2018). Discovery of the viral eccDNA in fruit flies and mosquitoes opens up a new avenue to investigate the biogenesis and function of the vsiRNAs. Diverse positive- and negative-strand viral RNAs produced during either the viral RNA replication cycle, including DVG RNAs, or the host antiviral RNAi (Figure 1), may serve as the templates for host reverse transcriptase (RTase), which may account for the accumulation of the genome-wide vsiRNAs. Production of the primary vsiRNAs is necessary to trigger the host RdRP-dependent biogenesis of the secondary vsiRNAs in both plants and nematodes (Ding, 2010). However, it is currently unknown whether production of the vsiRNAs templated by the viral eccDNA occurs after or in parallel with the biogenesis of the primary vsiRNAs processed from viral dsRNA replicative intermediates (Figure 1). Plant primary and secondary vsiRNAs are biochemically indistinguishable because both are made by Dicer (Ding, 2010). In contrast, nematode secondary vsiRNAs are Dicer independent, predominantly 22 nt in length, and antisense with 5′ triphosphates, and the fly vsiRNAs in the exosome-like vesicles also contain 5′ triphosphates (Tassetto et al., 2017). Thus, further studies are necessary to determine whether the vsiRNAs templated by the viral eccDNA contain 5′ triphosphates in addition to 5′ monophosphates cloned by Poirier et al. (2018). Lastly, infection of mature mouse, monkey, and human cells with distinct RNA viruses also triggers Dicer-mediated production of the vsiRNAs with similar abundance to the insect vsiRNAs (Li et al., 2013, 2016; Qiu et al., 2017). As mammals do not encode a protein homologous to the plant and nematode RdRPs, whether mammals use a similar strategy to boost the abundance of vsiRNAs via the production of viral eccDNA is a critical question that remains to be addressed.

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A Sleeping Area of Malaria Research Awakes

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https://doi.org/10.1016/j.chom.2018.02.008

Phenotypic screening methods have had a profound impact on antimalarial drug development, but assays that predict which compounds might provide a radical cure have remained elusive. In this issue of *Cell Host & Microbe*, Gural et al. (2018) report hypnozoite culturing and systems to study these elusive, yet deadly, parasites.

The holy grail in the antimalarial drug development field would be a new drug that can provide a radical cure for certain types of malaria. In this issue of *Cell Host & Microbe*, Gural et al. (2018) describe important progress toward setting up a phenotypic assay that can predict which drug-like compounds might be able to provide a radical cure and are thus worthy of further investment.

If you have ever donated blood, you may have been asked if you have ever had malaria, and if you answered "yes," you might be turned away even if you had the disease many years ago. The reason for this question comes from the fact that some species of malaria parasite, including the human parasites *Plasmodium vivax* and *P. ovale*, as well as the monkey parasite *P. cynomolgi*, can hide quiescently in the liver for