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Broad RNA Interference–Mediated Antiviral Immunity and Virus-Specific Inducible Responses in Drosophila

Cordula Kemp,*1 Stefanie Mueller,*1,2 Akira Goto,* Vincent Barbier,* Simona Paro,* François Bonnay,* Catherine Dostert,† Laurent Troxler,* Charles Hetru,* Carine Meignin,* Sébastien Pfeffer,† Jules A. Hoffmann,* and Jean-Luc Imler*‡

The fruit fly Drosophila melanogaster is a good model to unravel the molecular mechanisms of innate immunity and has led to some important discoveries about the sensing and signaling of microbial infections. The response of Drosophila to virus infections remains poorly characterized and appears to involve two facets. On the one hand, RNA interference involves the recognition and processing of dsRNA into small interfering RNAs by the host RNase Dicer-2 (Dcr-2), whereas, on the other hand, an inducible response controlled by the evolutionarily conserved JAK-STAT pathway contributes to the antiviral host defense. To clarify the contribution of the small interfering RNA and JAK-STAT pathways to the control of viral infections, we have compared the resistance of flies wild-type and mutant for Dcr-2 or the JAK kinase Hopscotch to infections by seven RNA or DNA viruses belonging to different families. Our results reveal a unique susceptibility of hop mutant flies to infection by Drosophila C virus and cricket paralysis virus, two members of the Dicistroviridae family, which contrasts with the susceptibility of Dcr-2 mutant flies to many viruses, including the DNA virus invertebrate iridescent virus 6. Genome-wide microarray analysis confirmed that different sets of genes were induced following infection by Drosophila C virus or by two unrelated RNA viruses, Flock House virus and Sindbis virus. Overall, our data reveal that RNA interference is an efficient antiviral mechanism, operating against a large range of viruses, including a DNA virus. By contrast, the antiviral contribution of the JAK-STAT pathway appears to be virus specific. The Journal of Immunology, 2013, 190: 650–658.

Viruses represent an important class of pathogens, causing serious concern for human health, as well as important economic losses in crops and animals. Because they replicate inside cells, and rely for the most part on host cell molecular machineries for their replication, viruses pose specific challenges to the immune system. Two major strategies of antiviral resistance have been described. In mammals, viral infection is first detected by pattern recognition receptors of the Toll- and RIG-I-like families that sense the viral nucleic acid and trigger the induction of IFNs and other cytokines (1). These factors activate the production of antiviral molecules, such as protein kinase R or oligo-2', 5'-adenylate synthetase, that contain the infection and contribute to the activation of the adaptive immune response (2). In plants, viral nucleic acids are recognized by enzymes of the Dicer family, which produce small interfering RNAs (siRNAs) of 21–24 nucleotides. These siRNAs are then loaded onto molecules of the Argonaute (AGO) family and will guide them toward RNAs with complementary sequences; targeted RNAs are then either sliced by AGO, or their translation is inhibited. This RNA interference (RNAi) mechanism provides efficient and sequence-specific protection against viral infections (3).

RNAi also plays an important role in the control of viral infections in insects, as shown by the production of virus-derived siRNAs in infected flies, and the increased susceptibility to viral infection of Drosophila mutants for the genes Dcr-2 and AGO2 (3–6). In addition, several reports indicate that an inducible response also contributes to the control of viral infections (7–15). We previously showed that infection with Drosophila C virus (DCV), a member of the Dicistroviridae family, leads to induction of some 130 genes (11). Analysis of the regulation of one of these genes, vir-1, revealed the presence of functionally important binding sites for the transcription factor STAT in its promoter. The induction of vir-1, as well as several other DCV-induced genes, was found to be dependent on the gene hopscotch (hop), which encodes the only JAK kinase in Drosophila. Furthermore, hop mutant flies succumb more rapidly than do wild-type controls, with a higher viral load, to DCV infection (11). The Toll and immune deficiency (Imd) pathways, initially characterized for their role in the control of bacterial and fungal infections, were...
also thought to play a part in the control of viral infections. Whereas the Toll pathway was associated with resistance to the *Drosophila* X virus (DXV) (15), the Imd pathway was implicated in the control of Sindbis virus (SINV) (7) and cricket paralysis virus (CrPV) (9).

Altogether, the data in the present literature point to the involvement of both RNAi and an inducible expression of effector molecules to counter viral infections in insects (5, 16). However, whereas RNAi was shown to contribute to resistance to several RNA viruses (with either single-stranded genomes of both polars or double-stranded genomes), most studies on the inducible response have so far focused on a single virus. As a result, the global significance of the inducible response for the control of viral infections remains poorly understood. In particular, it is unclear at present if the JAK-STAT pathway is involved in a general antiviral response, providing broad antiviral immunity, or if it acts specifically on a critical step in the replication cycle of a specific virus or virus family. To address this important question, we have compared the resistance of a mutant for the JAK-STAT pathway to infection by seven RNA or DNA viruses. We find that *hop* mutant flies are more susceptible than wild-type controls to infection by the Dicistroviridae DCV and CrPV, but exhibit either no or a weak phenotype for other viruses, suggesting that the JAK-STAT pathway–inducible response is virus specific. Genome-wide transcript profiling shows that infection by two other RNA viruses, Flock House virus (FHV, *Nodaviridae*) and SINV (*Alphaviridae*), leads to upregulation of ≥400 genes, which only partially overlap with those induced by DCV. Overall, our data indicate that the siRNA pathway exerts broad antiviral activity and affects both RNA and DNA viruses, with virus-specific inducible responses contributing to the control of viral infections in *Drosophila*.

**Materials and Methods**

**Fly strain culture and infection**

*Oregon-R (OR)* and *yw* were used as wild-type control flies. The *hop* strain was previously described (17–19). A genomic rescue of the *Dcr-2* gene was established with the Fonsoid *Fly* Fox017074 (transgeneome.mpi-cbg.de) inserted at the landing site atp40 (2L), and the transgenic chromosome was recombined with the landing site OR. 

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Assembly, sequencing, and analysis of small RNA libraries

The small RNA library of S2 cells and whole flies was constructed as described (22) and sequenced by the Illumina 2G Analyzer. Reads were then aligned to a reference consisting of the IIV-6 genome from the National Center for Biotechnology Information (accession code NC_003038) using the Bowtie program with standard parameters in genome assembly. Reads aligning to the IIV-6 genome with a maximum of one mismatch were retained and analyzed using in-house Perl scripts and Excel. Sequences were submitted to the National Center for Biotechnology Information Small Read Archive (http://www.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?) under the accession number GSE41007.

Results

RNAi provides broad antiviral protection in Drosophila

Several independent studies, including our own, have established that RNAi, and more precisely the siRNA pathway, serves as an efficient host defense against RNA viruses. These include viruses with a single-stranded genome of both (+) and (−) polarity and dsRNA viruses (23–30), and we confirmed that flies mutant for Dcr-2 died more rapidly than wild-type controls when they were infected with DCV, CrPV, FHV, SINV, VSV (Rhabdoviridae), and DXV (Birnaviridae) (data not shown). Next, we addressed the question whether the siRNA pathway also participated in the control of a DNA virus infection, and infected wild-type and RNAi mutant flies with IIV-6 (Iridoviridae). Infection of Dcr-2 mutant flies led to a more rapid and intense appearance of blue color, which is characteristic of the accumulation of iridescent viral particles, than in wild-type controls (Fig. 1A). Dcr-2^{2/416X} flies were significantly more susceptible to IIV-6 infection than were the corresponding wild-type (Fig. 1B). A fraction of Dcr-2^{2/416X} flies injected with buffer also died in the course of the experiment, confirming the increased sensitivity to stress associated with mutations of the siRNA pathway (31). The decreased survival time correlated with a 20-fold increased viral load in Dcr-2 mutant flies at 10 d postinfection (dpi) (Fig. 1C). Similar results were obtained when a different null allele of Dcr-2 was used, and the IIV-6 susceptibility phenotype was rescued by a wild-type genomic Dcr-2 transgene (Fig. 1D). The r2d2^{2/4} and AGO2^{2/4} null mutant flies also exhibited increased sensitivity to IIV-6 (Fig. 1E). AGO2^{2/4} flies contained more viral DNA than did wild-type controls, confirming that this gene participates in the control of infection (Fig. 1F).

We next sequenced small RNA libraries prepared from IIV-6–infected S2 cells or adult flies. We observed several hundreds of thousands of reads matching the IIV-6 genome in both infected S2 cells and wild-type flies, but not in control noninfected S2 cells (Supplemental Table I). The large majority of these reads had a size of 21 nucleotides, which is characteristic for processing by the RNase Dicer-2 (Dcr-2). This peak was absent from the library prepared from infected Dcr-2^{2/416X} mutant flies (Fig. 2A). These data indicate that Dcr-2 generates 21-nucleotide IIV-6–derived siRNAs in infected flies, and raise the question of the nature of the substrate used by Dcr-2 in the context of this infection. As previously reported for RNA viruses, the number of reads matching

![Image](http://www.jimmunol.org/Downloadedfromhttp://www.jimmunol.org/)
each strand of the viral genome was very similar (Supplemental Table I). However, unlike RNA viruses, the virus-derived siRNAs were not uniformly distributed along the viral genome. Rather, several hotspots were observed, revealing that specific regions of the viral genome generate the siRNAs (Fig. 2B, 2C). These peaks do not correlate with the intensity of transcription of the viral genome, and some highly transcribed regions are located in areas not generating significant levels of siRNAs (32). The strong symmetry of the peaks observed in S2 cells and wild-type flies suggests that these regions are transcribed on both strands and generate dsRNA. Indeed, we could detect bidirectional transcription in the areas of the viral genome covered by the peaks (Fig. 2D). By contrast, transcription of only one strand of the DNA genome was detected for the locus 261R, which is located in a region that does not produce significant amounts of siRNAs. Overall, these results indicate that the siRNA pathway in Drosophila can also protect against a DNA virus infection.

The JAK kinase Hopscotch does not confer broad antiviral immunity

To test the contribution of the JAK-STAT pathway in antiviral immunity in Drosophila, we injected loss-of-function mutants of the JAK kinase Hopscotch (hop<sup>M380<sub>mut</sub></sup>) with different ssRNA, dsRNA, and DNA viruses. As previously described, hop<sup>M380<sub>mut</sub></sup> mutant flies die more rapidly than do wild-type controls following DCV infection, and contain ~10-fold more virus (Fig. 3A). By contrast, we did not observe significant differences in survival between wild-type and hop<sup>M380<sub>mut</sub></sup> mutant flies upon infection with the alphavirus SINV (Fig. 4A), and the viral titers 2 dpi were not significantly different in wild-type and hop<sup>M380<sub>mut</sub></sup> mutant flies (data not shown), indicating that the JAK-STAT pathway does not contribute to resistance to this virus. The hop<sup>M380<sub>mut</sub></sup> mutant flies, as well as wild-type flies, also resisted infections by the rhabdovirus VSV and by the nodavirus FHV (Fig. 4B, 4C). A slight reduction in survival was observed in the case of the dsRNA virus DXV (Birnaviridae) and the DNA virus IIV-6 (Fig. 4D, 4E). However, the difference between wild-type and hop<sup>M380<sub>mut</sub></sup> mutant flies was only statistically significant in the case of DXV infection. Furthermore, we did not observe statistically significant differences in the DXV and IIV-6 viral titers in wild-type and hop<sup>M380<sub>mut</sub></sup> mutant flies in the format of our assays (data not shown).

Overall, our data indicate that the JAK-STAT pathway is critical for host defense against DCV, but plays a minor role for DXV and IIV-6 and is essentially dispensable in the case of FHV, SINV, and VSV. We therefore tested CrPV, another member of the Dicistroviridae family known to infect Drosophila. We observed a decrease in survival and a significant increase in viral titers in CrPV-infected hop<sup>M380<sub>mut</sub></sup> mutant flies compared with wild-type flies (Fig. 3B). In conclusion,

**FIGURE 2.** Virus-derived siRNAs in S2 cells and Drosophila adult flies infected by the DNA virus IIV-6. RNA was extracted 5 dpi from S2 cells infected by IIV-6 (MOI 0.01) and adult wild-type (yw) or mutant (Dcr-2<sup>204G6L</sup>) flies injected with IIV-6 (5000 PFU per fly). (A) Size distribution of the small RNAs matching the viral genome in S2 cells and adult flies of the indicated genotype. (B and C) Distribution of the 21-nucleotide siRNAs from the S2 cell (B) and yw adult fly (C) libraries along the IIV-6 genome. Each IIV-6–derived small RNA is represented by the position of its first nucleotide. The IIV-6–derived small RNAs matching the upper and lower strand of the DNA genome are respectively shown above (positive reads number) and below (negative reads number) the horizontal axis, which represents the 212482bp genome. In (B), the number of reads for four peaks going off-scale is indicated next to them, in italics. (D) Strand-specific RT-PCR with primers corresponding to the annotated viral genes 206R, 224L, 244L, and 261R. The experiment was performed in the presence (+) or absence (−) of RT. NI, Noninfected; F and R, forward and reverse strand primer used for reverse transcription.
our data indicate that the JAK-STAT pathway in *Drosophila* confers protection against some viruses—in particular, the *Dictyoviridae*—but does not provide broad antiviral immunity.

**Inducible gene expression in FHV- and SINV-infected flies**

The above results raised the question of whether an inducible response contributes to host defense against viruses other than DCV and CrPV. We therefore conducted a genome-wide microarray analysis using Affymetrix DNA microarrays to monitor gene expression in flies infected by FHV (2 and 3 dpi) or SINV (4 and 8 dpi), and compared the data with those obtained for DCV infection (1 and 2 dpi). The time points for this analysis were chosen to take into account the different kinetics of replication and colonization of *Drosophila* by the different viruses (11, 24). For each virus, we observed a large overlap between the genes induced at the first and second time points. We then pursued our analysis, focusing on the genes induced either at the first or at the second time point. The microarray data revealed that 487 and 201 genes were induced or expressed in flies infected by FHV (2 and 3 dpi) or SINV (4 and 8 dpi), and compared the data with those obtained for DCV infection (1 and 2 dpi), the time points for this analysis were chosen to take into account the different kinetics of replication and colonization of *Drosophila* by the different viruses (11, 24). For each virus, we observed a large overlap between the genes induced at the first and second time points. We then pursued our analysis, focusing on the genes induced either at the first or at the second time point. The microarray data revealed that 487 and 201 genes were induced or expressed in flies infected by FHV (2 and 3 dpi) or SINV (4 and 8 dpi), and compared the data with those obtained for DCV infection (1 and 2 dpi), the time points for this analysis were chosen to take into account the different kinetics of replication and colonization of *Drosophila* by the different viruses (11, 24). For each virus, we observed a large overlap between the genes induced at the first and second time points. We then pursued our analysis, focusing on the genes induced either at the first or at the second time point. The microarray data revealed that 487 and 201 genes were induced or expressed in flies infected by FHV (2 and 3 dpi) or SINV (4 and 8 dpi), and compared the data with those obtained for DCV infection (1 and 2 dpi), the time points for this analysis were chosen to take into account the different kinetics of replication and colonization of *Drosophila* by the different viruses (11, 24). For each virus, we observed a large overlap between the genes induced at the first and second time points. We then pursued our analysis, focusing on the genes induced either at the first or at the second time point. The microarray data revealed that 487 and 201 genes were induced or
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upregulated by a factor of at least 2 upon infection by FHV and SINV, respectively. When analyzed with the same criteria, 166 genes were induced by DCV (Fig. 5A, Supplemental Table II).

The data of this transcriptomic analysis call for two comments. First, we note that 42 genes were induced by all three viruses (Fig. 5A). We compared this set of genes with microarray studies performed on flies infected by fungi and bacteria (both extra- and intracellular) to identify a potential signature specific for viral infections (Supplemental Table III). We observed that a number of genes, such as Frost, are upregulated similarly by all types of infections, suggesting that they are induced by the stress of the infection, rather than by recognition of specific characteristics of the infecting microorganism. Of interest, other genes, such as Obp99b, Mal-B1, Gsta1, CG8147, CG1572, l(2)gd1, CG14906, CG10911, and Tsp42EI, appear to be induced only in response to viral infections, and may represent the core of an inducible antiviral gene expression program. The case of Obp99b is particularly striking, as this gene is strongly upregulated by FHV, SINV, and DCV, but inhibited following other types of infection. Clearly, the regulation and function of this molecule deserves further investigation. The genes CG4680, Eip75B, Sp7, and CG10916 are induced both by the viruses and by the intracellular bacterium Listeria (33), suggesting that they may participate in the defense against intracellular intruders (Supplemental Table III).

A second comment is that the majority of upregulated genes are induced by only one or two of the viruses, revealing virus-specific responses. Of interest, 84% of the genes upregulated by SINV are also induced by FHV, pointing to a strong similarity between the responses to the two viruses. FHV induced a higher number of genes than did Sindbis virus, and only 34% of the genes induced by FHV are also induced by SINV (Fig. 5A). It is intriguing, though, that many of the genes induced solely by FHV, but not by SINV, are members of the same gene families as the genes coinduced by both FHV and SINV. This peculiarity underlines the basic similarities between the transcriptional response to the two viruses. In addition, several genes associated with cell death are induced by FHV, but not SINV, which may reflect the higher virulence of FHV (Fig. 5B, Supplemental Tables II, III). Only 22% and 16% of the genes induced by SINV and FHV, respectively, are also induced by DCV, indicating that DCV, on one hand, and FHV and SINV, on the other hand, trigger different inducible responses (Fig. 5A). We did not detect in our microarrays expression of the genes encoding the unpaired (Upd) cytokines, which activate the JAK-STAT pathway in Drosophila. However, quantitative RT-PCR analysis revealed that upd2 and upd3, but not upd1, are induced or upregulated following DCV and CrPV infection (Fig. 3C).

Virus-specific pattern of gene induction

To further characterize the transcriptional response triggered by different viruses, wild-type flies were injected with DCV, CrPV, FHV, SINV, VSV, DXV, and IVV-6, and gene induction was measured at 6 h postinfection and 1, 2, 3, and 4 dpi. Gene expression was monitored by quantitative RT-PCR, which provides a more accurate quantification of gene expression than does hybridization to short oligonucleotide probes on microarrays (34). We monitored expression of the DCV-induced gene vir-1 (11) and of TotM, which, according to the microarrays, is induced by FHV and SINV infection. We confirmed the induction of vir-1 by DCV and FHV (11) and detected a milder but significant induction of this gene by CrPV infection. By contrast, no induction of vir-1 by SINV, VSV, DXV, and IVV-6 was observed (Fig. 3C). For TotM,
we confirmed the induction by FHV at different time points. In addition, we observed that TotM expression was significantly induced by DCV at late time points of infection (4 dpi). We note that induction of TotM by SINV, VSV, and DXV was 10–20 times stronger than the induction by FHV (Fig. 5C). The DNA virus IIV-6 did not induce TotM at any measured time point. Interestingly, we observed different profiles for vir-1 and TotM induction after viral challenge. Overall, the viruses that kill wild-type flies severely (within 10 d), such as DCV, CPV, and FHV, were potent inducers of vir-1, whereas less pathogenic viruses, such as SINV, VSV, and DXV, did not induce vir-1. The opposite trend was observed for TotM, which was most potently induced by SINV, VSV, and DXV, and did not induce vir-1. The opposite trend was observed for TotM, which was most potently induced by SINV, VSV, and DXV, and did not induce vir-1. The different pattern of induction of vir-1 and TotM suggests that the two genes may be regulated differently, even though both were previously shown to be regulated by the JAK-STAT pathway (11, 17). Indeed, the MAP3K MEK kinase 1 (MEKK1) and the Imd pathways are also known to contribute to the induction of TotM induction in some contexts (17, 35).

Some antimicrobial peptide genes were also upregulated according to the microarrays, suggesting an overlap between antiviral immunity and antibacterial–antifungal defenses. We observed an enrichment for genes regulated by the Toll pathway [e.g., the cytokine Spaezle (Spx) and the antifungal peptides Drosomycin (Dr3) and Metchkinikowie (Mtk)] in the DCV-specific set of genes (Supplemental Table II). We also noted an enrichment of Imd pathway–regulated genes, such as the antibacterial peptides Attacin-A and -C, Diptericin-B, and the transcription factor Relish, in the genes upregulated by both DCV and FHV. However, when expression of dptiercin and drosomycin—two markers of activation of the Imd and Toll pathways, respectively—was monitored by quantitative RT-PCR, none of the viruses triggered an induction comparable to that of bacterial and fungal infections, although the wounding associated with the injection procedure clearly led to some expression of the genes (Supplemental Fig. 1).

Discussion

We have investigated the involvement of RNAi and the evolutionarily conserved JAK-STAT signaling pathway in the resistance to a panel of seven viruses representing several important families, including the arboviruses SINV and VSV. Our data provide a contrasting picture: on the one hand, a broad antiviral immunity based on RNAi contributing to the defense against both RNA and DNA viruses, and on the other hand, a virus-specific transcriptional response involving the JAK-STAT pathway but playing a critical role only in the case of Dicistroviridae infection.

RNAi protects against a DNA virus infection

The present study extends work from several groups, including our own, showing that flies mutant for the siRNA pathway are more sensitive than wild-type flies to a large panel of RNA viruses, and reveals that Dcr-2 is also required for the control of the DNA virus IIV-6. We note, however, that the increase of viral titer in RNAi–deficient flies is not as strong as in the case of some RNA viruses [e.g., VSV (25)]. This finding could reflect either the expression of a viral suppressor of RNAi by IIV-6 or the fact that only a portion of the viral genome is targeted by siRNAs. Indeed, this virus encodes an RNaseHII enzyme, which could cleave siRNA duplexes, as previously reported in plants infected by the sweet potato chlorotic stunt virus (36). The involvement of Dcr-mediated immune responses against DNA virus infections was previously noted in plants, in which secondary structures in the transcribed viral RNAs, or dsRNAs formed from overlapping bidirectional transcripts, can be processed into siRNAs (37, 38). Production of dsRNA from DNA viruses also occurs in animal cells, as demonstrated by the critical role played by the dsRNA receptor TLR3 in the sensing of herpesvirus infection in mammals (39, 40). Our data are consistent with a model whereby dsRNA generated from convergent transcription of the IIV-6 genome is processed by Dcr-2 and triggers RNAi. Thus, we conclude that RNAi provides an efficient and highly specific RNA-based defense against many types of viruses in Drosophila and probably other insects. This conclusion parallels the situation described in plants. The vertebrates, which largely rely on the induction of IFNs to counter viral infections, appear to be the exception among multicellular organisms (1). Of interest, however, the DEXD/H box helicase domains found in Dcr enzymes and RIG-I–like receptors, which sense the presence of viral RNAs in cells infected by RNA and DNA viruses, are phylogenetically related (10). This finding suggests that an essential domain of a core molecule from the ancestral antiviral response, RNA silencing, was at some point recruited to sense viral RNAs in vertebrates and to subsequently activate a signaling pathway leading to production of IFNs.

Virus-specific induced gene expression in Drosophila

Microarrays are powerful tools to monitor the global transcriptome of infected cells and compare the response to different infections. Despite its limitations for accurate measurements of the magnitude of expression changes, this technology provides useful information on changes in gene expression (34). In this article, we used whole-genome Affymetrix microarrays to analyze the transcriptome of flies infected by DCV, FHV, or SINV, we report the existence of virus-specific responses to infection. These results are in keeping with a previous study pointing toward a polyphagous as an antiviral defense mechanism against VSV, but not DCV, infection (14). The three viruses we used belong to different families and present different characteristics that make them valuable for the current study. For example, 1) DCV and FHV replicate rapidly and kill Drosophila upon infection, whereas SINV does not at the dose used (11, 24); 2) DCV is a natural pathogen of Drosophila, whereas FHV and SINV have not been found in wild Drosophila populations (41); 3) FHV and DCV possess, respectively, a strong and moderate viral suppressor of RNAi, whereas SINV presumably does not (28, 42, 43). The three viruses also have different tissue tropism and may be associated with tissue-specific modifications in the physiology of the infected host. For example, FHV was recently shown to be a cardiotropic virus, affected by potassium channels regulating heart function (44), whereas DCV infection causes intestinal obstruction (S. Chitarbanova and J.-L. Imler, manuscript in preparation).

Comparison of the transcriptomes of the flies infected by the three viruses revealed more similarities between FHV and SINV than between each of these and DCV. This may reflect the co-evolution of DCV with its host, and the fact that this virus may have learned to ward off the antiviral arsenal of its host. Indeed, DCV induces fewer genes than does FHV, even though the two viruses replicate with similar kinetics and lead to the rapid death of the flies. The genes induced by FHV and SINV encode chaperonins (Tcp or Hsp), glutathione transferases, cytochrome P450s, stress markers (Tot family), thioester-containing proteins, and cytoskeletal regulators, suggesting an involvement of oxidative stress and phagocytosis in the response to these viruses. The two viruses also upregulate the gene egghead (egh), which encodes a molecule involved in the uptake of dsRNA and antiviral immunity (27). Despite the large overlap between the genes upregulated by FHV and SINV, the former induce a more intense transcriptional response than the latter. This observation may reflect the more aggressive replication of FHV in Drosophila. Indeed, the genes specifically induced by FHV include not only additional members of the families mentioned above (Hsp, Tcp, Gst, cytP450, thioester-
An unexpected finding reported in this article is that Dicistroviridae activators of chemoattraction of phagocytic cells (53).

As flies mutant for either RNAi or the inducible JAK-STAT pathway succumb to infection 2–3 d before the controls, with an ~10-fold increase in viral titer.

Interestingly, even though hop mutant flies appear to be specifically sensitive to Dicistroviridae, other viruses activate the JAK-STAT pathway. Indeed, we observed a slight increase in the lethality of hop mutant flies postinfection with DXV and IVV-6. In Aedes mosquitoes, the JAK/STAT pathway was also shown to activate a defense against Dengue, a member of the Flaviviridae family (54). We also note that the JAK-STAT pathway–regulated gene vir-1 (11) is induced by DCV and CrPV, but also FHV, even though hop mutant flies resist FHV infection much as do wild-type flies. One hypothesis to explain this apparent paradox is that some genes may be induced in a JAK-STAT–independent manner in the context of viral infections. For example, the gene TotM, which is induced by several viruses normally resisted by hop mutant flies, can be induced by the MEKK1 pathway, in addition to the JAK-STAT pathway (35). Indeed, we observed that TotM remains fully induced by FHV and SINV in hop mutant flies (C. Dostert and J.-L. Imler, unpublished observations). However, this hypothesis cannot account for the induction of vir-1 by FHV, because it is strongly reduced in hop mutant flies (C. Dostert and J.-L. Imler, unpublished observations). This finding suggests that some aspects of the JAK-STAT–induced response may be redundant of other defenses for FHV, but not for DCV. The fact that FHV triggers a stronger transcriptional response than does DCV (Fig. 5) is consistent with this hypothesis.

A key question pertains to the nature of the receptor detecting Dicistroviridae infection and triggering the JAK-STAT–dependent inducible response. Our data point to the induction of a specific subset of genes, including the JAK-STAT–regulated gene vir-1 (11), by fast-killing viruses such as DCV and CrPV, but also FHV, which replicate rapidly to high titers upon injection in flies. Of note, vir-1 induction is not affected in flies expressing the dsRNA-binding protein B2, or in Dcr-2 mutant flies, indicating that this gene is not induced following sensing of dsRNA (10). This finding suggests that sensing tissue damage and/or cell death could contribute to this inducible response, a hypothesis corroborated by the association of the JAK-STAT pathway with the cellular response to a variety of stresses (17, 55–57).

In conclusion, our data confirm that, beyond RNAi, an inducible response contributes to the control of some viral infections in Drosophila. However, this response is complex, and great care should be exercised before generalizing the results obtained with one single virus species. This unexpected complexity probably reflects the intricate association of viruses with their host cells in different tissues, their different strategies of replication or protein expression, or their acquisition of suppressors of host defense.

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Disclosures
The authors have no financial conflicts of interest.