Published in final edited form as:

Curr Opin Microbiol. 2008 June ; 11(3): 262–270. doi:10.1016/j.mib.2008.05.007.

Genomic RNAi screening in *Drosophila* S2 cells: What have we learned about host-pathogen interactions?

Sara Cherry

Department of Microbiology, Penn Genomics Frontiers Institute, University of Pennsylvania, 19104

Abstract

The détente between pathogen and host has been of keen interest to researchers in spite of being exceedingly difficult to probe. Recently, new RNA interference (RNAi) technologies, in particular in *Drosophila* tissue culture cells, have made it possible to interrogate the genetics of host organisms rapidly, with nearly complete genomic coverage and high fidelity. Therefore, it is not surprising that the applications of RNAi to the study of host-pathogen interactions were amongst the first to be published, and have already revealed many new insights into the hosts' role in infection. This review will highlight the application of RNAi screening to pathogen-host interactions in *Drosophila* cells and will reveal some of the lessons learned from this approach.

Introduction

All organisms are plagued by infectious pathogens including bacteria, fungi, parasites and viruses. In order to successfully invade and survive within their hosts, pathogens must exploit cellular pathways and establish their particular niche, as well as evade detection by the host-encoded immune system. Pathogens must also remodel and subvert host pathways to facilitate their own survival at the expense of the host. Viruses, as obligate intracellular pathogens with only a limited genome size, are even more dependent on host encoded factors for their replication cycle. The identification of host encoded factors involved in infection, intracellular replication and pathogenesis has historically been difficult due to the dearth of pathogen-host systems amenable to genetic screening, as well as the lack of a suitable *in vivo* model to test the interactions between the pathogen and host. To rapidly identify host encoded factors that affect microbial pathogenesis, recent studies have taken advantage of the genetically tractable *Drosophila* model system. Practical aspects of the *Drosophila* system enable unbiased approaches to the identification of host-encoded factors that impact the pathogen-host interface both at the cellular and organismal level.

Over the past several decades, studies in *Drosophila* have been central to our increasing understanding of various fundamental biological processes [1–3]. *Drosophila* have conserved developmental and cell biological processes making them a fruitful model for mammalian development and disease [4]. Moreover, many of the classic signal transduction systems were identified first in *Drosophila* using forward genetic screens. Because the *Drosophila* genome is compact with relatively low redundancy, single mutants are likely to reveal phenotypes of interest, in contrast to mammals where redundant gene families can make genetic analysis more

cherrys@mail.med.upenn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

complex. Recently, many insights into innate immunity have been obtained from both forward and reverse genetic studies in *Drosophila* [5–9]. *Drosophila* has no acquired immune system, and so relies exclusively on the innate immune system. The impact of *Drosophila* studies on our general understanding of innate immunity is underscored by the discovery and characterization of the single-pass, transmembrane-receptor Toll and its mammalian homologues, the Toll-like receptors, which play critical roles in innate immune responses to pathogens in both insects and mammals. Because of this conservation, and the ease of performing large-scale genetic screens, *Drosophila* has been instrumental in uncovering new connections between cellular factors, innate immunity and pathogens.

Host-Pathogen Interactions and Innate Immunity

The two main host-encoded processes that should be considered when discussing the interactions between pathogens and hosts are the host-encoded immune system used to combat the pathogen and the cellular factors used and/or manipulated by the pathogen for its survival. It is essential to understand both pathogen virulence, and the host immune response, and both can be studied in the *Drosophila* system. The *Drosophila* innate immune system involves both cellular and humoral responses, with both cell intrinsic and extrinsic components. The cellular arm of the innate immune system involves both specialized immune cells which recognize and respond to invaders as well as intrinsic immune mechanisms initiated within an infected cell. *Drosophila* have specialized professional phagocytic cells with properties similar to mammalian macrophages. These cells efficiently ingest particles and microbes and upon stimulation produce cytokines that modulate the downstream immune response. Although these specialized immune cells are an essential component of the innate immune response [9], it has become increasingly clear that all infected cells have some intrinsic capacity to respond to infections. A classical example is the production of interferons which can be produced by most virally-infected mammalian cell-types. In *Drosophila* these cell-intrinsic responses include apoptosis, autophagy and RNAi.

In *Drosophila*, the humoral arm of the innate system is characterized by the production of secreted factors that are produced by the activation of a variety of conserved signaling pathways to control infection. These cascades are activated downstream of pathogen recognition. Rather than specifically recognizing individual antigens unique to each pathogen as B- and T-cell Receptors do, the innate system responds to pathogen-associated molecular patterns (PAMPs)—motifs that are essential functional components of microorganisms, and are thus broadly expressed across classes of pathogens [10]. *Drosophila* uses many of the same recognition receptors and signaling pathways as mammals to initiate these anti-microbial cascades. For example, scavenger receptors, TLRs as NFkB signaling are essential components of both mammalian and insect responses.

The other essential component of the host-pathogen interface is the factors that are subverted by the microbe to allow for its survival and replication. *Drosophila* uses highly conserved cell biological processes, but has less functional redundancy than mammals and other vertebrates. Thus, the use of genetic loss-of-function approaches to uncover the role of basic cellular functions in microbial pathogenesis is potentially more fruitful in *Drosophila* than in mammals. This is particularly important for intracellular pathogens and allows for the elucidation of pathways involved in intracellular growth of the invader. Pathogens are efficient cell biologists, reprogramming pathways and thereby teaching us both about the pathogen's requirements and about essential processes at work in uninfected cells (i.e. splicing [11], transcription factors [12], etc. all discovered via studying virus infection). Importantly, because some host factors are essential for microbial growth, they may prove to be useful targets for anti-microbial therapeutics.

Drosophila Cell Culture Screening platform

While some aspects of the host-pathogen interface require organismal study, others can be studied at the cellular level. Cellular pathogen recognition and the downstream signaling pathways are readily assayed in *Drosophila* cells. Moreover, tissue-specific responses can be probed using cell lines derived from disparate cell types. For example, hemocyte-derived cells such as Schneider line 2 (S2) cells as well as Kc167 cells are responsive to PAMPS and can thus be used to study this aspect of immunity. Moreover, these cell lines are highly phagocytic and therefore have been used to study pathogen uptake and clearance. Intrinsic innate immune mechanisms including anti-viral RNAi, autophagy, and programmed cell death can also be studied in cultured cells. For intracellular pathogens, host factors required for pathogen replication and survival can be identified. The use of cell culture also allows the role of proteins and processes that are essential early in development to be assessed for an independent role in immunity.

Importantly, *Drosophila* cells can be infected by a wide variety of pathogens: intracellular and extracellular, fungal, bacterial and viral. For example, RNAi has been used to study factors involved in host interactions with pathogens including: *Escherichia coli*, *Staphylococcus aureus*, *Mycobacterium sp.*, *Legionella pneumophila*, *Candida albicans*, *Chlamydia sp.*, *Listeria monocytogenes*, and *Drosophila C virus* (see below). Some of these are human pathogens while others are insect pathogens. The lessons learned from the study of these pathogens in *Drosophila* have revealed much about host-pathogen interactions and innate immunity in mammals. Table 1 presents an overview of the screens described in this review.

RNA interference in *Drosophila* cells is relatively efficient and straight-forward, and thus has been extensively used to study many aspects of biology that can be modeled in cells *in vitro* [13]. Each step in the process must be carefully selected and optimized. A flow chart of the process is schematized in Figure 1. Bathing of many *Drosophila* cell lines with naked double-stranded RNA (dsRNA) 200–500bp in length results in uptake, processing and gene knock-down in greater than 95% of the cells. This allows for the simple generation of arrayed RNAi libraries which can be used to probe for factors that, when depleted, impact a phenotype of interest. A variety of dsRNA libraries have been used, and are typically employed in a 384 well format allowing for high-throughput screening [14]. Different libraries have been used to dissect host factor requirements, ranging from focused gene-sets to genome-wide libraries (Figure 1). Most assays allow 3–5 days for the RNAi to take effect, yielding hypomorphic loss of function phenotypes. Assays must be designed to give robust and reproducible results; optimization for the 384-well format is particularly important. A number of different read-outs have been used ranging from microscope-based screens for microbial growth, to luciferase-based reporter gene assays for signaling pathways (See descriptions below). Each assay has been optimized to query specific steps in the biology of host-pathogen interactions. Knockdown of a given gene can either decrease or increase the read-out in question and potential positive candidates can be identified using statistical cut-offs or visual observation. Subsequent replication is used to filter out false positives and secondary assays are used to more fully characterize the role of the identified candidates in the biological process under study.

Anti-Microbial Signaling Screens

The systemic response to bacterial and fungal infection in *Drosophila* is mediated by two major signaling pathways that lead to the production of anti-microbial factors: the Toll pathway, which primarily responds to Gram-positive and fungal pathogens and the Immune deficiency (IMD) pathway, which largely responds to Gram-negative bacteria [6,7,15]. Another pathway that has known signaling roles in innate immunity and in particular in anti-viral immunity is

the JAK/Stat pathway [16–18]. Three large-scale RNAi screens have been performed, which probed the IMD signaling pathway in response to peptidoglycan [19–21] (Table 1). The first screen identified three classes of genes: positive regulators (49 DDRi genes), and two types of negative regulators: those that constitutively activated the NF- κ B reporter in the absence of LPS (26 CDRi genes) and those that enhanced the responsiveness to peptidoglycan induction (46 EDRi genes) [19]. The other two screens identified additional (but fewer) genes, including two new positive regulators of the signaling pathway, Iap2 and TAB [20,21]. Iap2 has been subsequently shown to be required for an immune response to gram-negative bacteria *in vivo* [22].

Two genome-wide screens have been performed to identify immune-associated components and mediators of the JAK/Stat signaling pathway using luciferase reporter systems [23,24] (Table 1). Muller et al identified 67 positive regulators and 24 negative regulators of the pathway while Baeg et al identified 29 positive regulators and 92 negative regulators. While the overlap of these screens is negligible, both studies focused on a novel phosphatase, PTP61F, that has been shown to be a bona fide negative regulator of signaling in *Drosophila* both *in vitro* and *in vivo*. The differences in cell type (Kc167 vs S2) or assay conditions (overexpressed cytokine vs endogenous) may explain the lack of concordance between the screens.

Microbial Recognition and Survival Screens

Recognition/Phagocytosis/Uptake

Phagocytosis is required for the uptake and clearance of intracellular microbes [25]. Phagocytosis is initiated by the ligation of cell-surface receptors that either directly bind to the particle or to opsonins that are deposited on the particle's surface. Professional immune cells such as hemocytes or hemocyte-derived S2 and Kc167 cells are particularly efficient at mediating uptake of a variety of microbes (and other particles), and have been used to screen for cellular factors required for phagocytosis.

To identify cellular mediators of phagocytosis of *E. coli* and *S. aureus*, 1000 randomly generated dsRNAs derived from an S2 cell cDNA library were screened using a FACS-based assay that monitored the uptake of FITC-labelled bacteria [26] (Table 1). 34 genes were required for efficient phagocytosis, but were not cytotoxic. Among these genes were four transcription factors, one of which encodes the master regulator of hematopoiesis in flies, the GATA-factor Serpent. Another gene, PGRP-LC, was required for efficient uptake of *E. coli* but not *S. aureus* in this system. Further studies showed that PGRP-LC is the peptidoglycan receptor for Gram-negative bacteria which in turn activates the IMD signaling pathway. Building on the identification of Serpent as an important factor required for efficient phagocytosis in S2 cells, Kocks et al performed expression profiling and identified 45 genes that were down-regulated by depletion of Serpent [27] (Table 1). Among those genes was SR-C1, a phagocytic scavenger receptor [28]. RNAi against these Serpent-dependent genes also identified a novel phagocytic receptor, Eater. This was the first member of a class of EGF-like-repeat-containing phagocytic receptors that act on a broad range of bacteria. Another member of this family, Nimrod, was also shown to mediate bacterial uptake and adhesion [29].

Candida albicans is a major nosocomial fungal pathogen of humans [30]. Using phagocytic S2 cells infected with GFP-expressing *Candida*, Stroschein-Stevenson et al. screened a library of 7,000 conserved genes for factors required for engulfment [31] (Table 1). They identified 184 genes that were required for efficient uptake. In particular, they identified Mcr (Macroglobulin-related protein), which is a member of a family of thioester-containing proteins, related to complement factors, called Teps, which act as opsonins. Mcr was shown to specifically opsonize *Candida* while TepII was required for efficient uptake of *E. coli* and

TepIII for *S. aureus*. This is the first functional evidence for a specific role of Tep proteins in *Drosophila* opsinization.

Another screen following *Mycobacteria fortuitum* infection identified 54 genes, including a novel class B scavenger receptor *peste* [32](Table 1). *Peste* was dispensible for *E. coli* and *S. aureus* uptake in S2 cells but was required for *L. monocytogenes* infection in addition to *M. fortuitum*. [33]. Although *Peste* was dispensible in S2 cells for uptake of *E. coli*, expression of *Peste* in non-phagocytic human cells was sufficient to support uptake of *E. coli* or *M. fortuitum*, suggesting that there may be a larger number of functionally redundant receptors for *E. coli* than for other bacteria such as *M. fortuitum* and *L. monocytogenes*.

Altogether, the results from these screens demonstrate that different classes of entry receptors mediate uptake of different microbes, sometimes with overlapping and redundant specificities. These RNAi screen have identified many of these receptors, suggesting that this aspect of immunity is particularly amenable to such approaches. The RNAi screens listed above, along with additional screens using live bacterial infection, also identified genes required for the downstream steps of phagocytosis including actin remodeling and membrane reorganization (Table 1). Many genes identified in *Drosophila* were previously known to be required for phagocytosis in mammalian systems, validating this approach. For example, an important role for actin remodeling, including the Arp2/3 complex, and vesicular transport genes was uncovered in most of these screens. Taking advantage of this knowledge, Pielage, et al screened ~80 genes known to regulate the actin cytoskeleton to identify additional factors essential for *Pseudomonas aeruginosa* entry [34]. This screen revealed an invasion pathway that is dependent upon the Abl signaling pathway both in *Drosophila* and mammalian cells.

In addition to known factors, RNAi screens were the first to identify the coatamer complexes (COPI and COPII) in uptake [26,31–33,35,36]. The COPI and COPII complexes are required for vesicular trafficking between the ER and golgi [37], are found to associate with the phagosome, and are required in S2 cells for the uptake of a wide variety of pathogens, although the mechanism of this requirement has not yet been established. Another novel and general finding of the RNAi screens was the requirement for the exocyst in phagocytosis [26,32,33, 35,36]. This complex plays essential roles in secretory vesicle targeting and docking at the plasma membrane for exocytosis [38]. And again, these factors associate with the phagosome, and were identified as essential components of the phagocytic machinery in a number of RNAi screens. Other cell biological and metabolic pathways were identified in a number of screens including fatty acid metabolism (e.g., [17,32,39]). However, further study is needed to determine whether these pathways are directly or indirectly involved in membrane dynamics of phagocytosis.

Bacterial Survival and Growth

Several other RNAi screens, using live intracellular bacteria, were designed not only to monitor uptake into host cells, but also intracellular replication and survival of the microbe. These screens have revealed additional cellular pathways and processes that impact these steps in the pathogen lifecycle. Each intracellular microbe subverts a unique cellular compartment to its own advantage. For example, *Listeria monocytogenes*, a gram positive bacterium, exits from a phagosome to replicate in the cytoplasm, while *Legionella pneumophila* and *Mycobacteria* species modify the phagosome, preventing phagosome-lysosome fusion [40]. Given the unique niche of each microbe, these screens have identified novel cellular factors thought to control intracellular survival, many of which are unique to each pathogen screened (Table 1).

Two independent screens were performed for factors involved in *L. monocytogenes* infection [33,35]. Agaisse et al identified 160 genes that blocked infection, of which a large number are thought to be involved in vacuolar escape, although this was not directly tested. Cheng et al

performed three screens. Screening with wild type *L. monocytogenes* they identified 89 genes required for infection; surprisingly, only a small subset of candidate genes overlapped with those found by Agaisse et al. In a parallel screen, they also assayed for factors that bypass the requirement for listeriolysin O (LLO), the bacterially-encoded enzyme required for exit from the phagosome, using an LLO-deficient *L. monocytogenes* strain. They identified 29 genes that specifically allow escape of this strain. In a third screen, the authors used a strain of *Listeria* carrying a mutant form of LLO with enhanced toxicity. This allowed the identification of 8 factors required for LLO toxicity. Among these genes were proteosomal components, which were also identified as required for infection in the Agaisse screen. Further experiments will be required to more fully clarify the roles of these factors in *Listeria* biology. Agaisse, et al also identified 48 genes that resulted in increased bacterial loads when targeted by RNAi, amongst which were a large number of cell cycle regulators. While this phenotype may be due to increased uptake, or increased bacterial growth, it is also possible that this group of genes were identified because their depletion was cytotoxic, leading to decreased cell number resulting in an increased multiplicity of infection (Table 1)

Mycobacterial species block phagosome-lysosome fusion in macrophages allowing for their replication and survival in that niche [41]. Two pathogenic species, *M. fortuitum* and *M. marinum* can infect and replicate in S2 cells. In addition the non-pathogenic strain *M. smegmatis* can invade but undergoes an abortive infection in S2 cells [32,42,43]. A genome-wide screen for factors required for *M. fortuitum* infection identified 32 genes that were required for survival but not uptake [32]. Amongst these genes were the ESCRT machinery and chromatin factors. In the absence of ESCRT (endosomal sorting complex required for transport) function, the phagosomal environment was altered resulting in differential bacterial growth whereby the non-pathogenic *M. smegmatis* strain is more successful, and the pathogenic *M. fortuitum* strain has altered gene expression profiles [44]. The ESCRT machinery was also identified as required for intracellular growth of *Listeria* suggesting that alterations in the phagosomal compartment may have more pleiotropic effects on intracellular bacterial survival [33]. A screen of 1000 dsRNAs to identify host factors that control *M. marinum* infection in S2 cells identified 2 genes that restrict infection [43]. Further studies demonstrated that one factor identified in the screen, lysosomal beta-hexosaminidase, restricts bacterial growth both in insect cells and mammalian macrophages. The mycobactericidal effect is specific to *M. marinum*, as *L. monocytogenes* and *S. typhimurium* were unaffected, and may have significant effects in limiting the initial mycobacterial infection of macrophages.

L. pneumophila, the gram negative agent of Legionnaires disease, is an intracellular pathogen that creates an intracellular vacuolar replication niche [45]. Much is known about the bacterial regulators of this compartment while less is known about the host factors required for its generation. Dorer et al used a visual assay that monitored the size and intensity of Legionella-GFP vacuoles along with a combination of single and double knock-downs of 73 trafficking genes in Kc167 cells to identify an essential network of early secretory genes required for bacterial replication [46]. Redundant activities required the use of double mutants (combinations of dsRNAs) which would be difficult to perform in mammalian systems, again pointing to the utility of *Drosophila* to uncover important cellular factors subverted by pathogens.

Chlamydia spp. are gram negative obligate intracellular bacteria of which three species cause disease in humans [47]. Whereas the human pathogen *C. trichomatis* can initiate infection in S2 cells, it cannot complete its lifecycle [48]. A focused screen for early events in the lifecycle included actin regulators and led to the identification of 28 factors including the Abl kinase and components of the PDGFR signaling pathway as essential for early steps in the *C. trichomatis* infection both in insect and mammalian cells [49]. *C. caviae*, a guinea pig pathogen, productively infects S2 cells and was used to screen a genome-wide library, leading to the

identification 31 genes that are specifically required for *C. caviae* replication [39]. Among these genes was the Tom complex, which regulates mitochondrial protein import [50] and is required both in *Drosophila* and mammalian cells for *C. caviae* but not *C. trachomatis* infection.

Viral screens

Viruses have been widely used as tools to probe cell biology. This is because they efficiently hijack and subvert cellular processes to remodel cells into viral production factories. This is accomplished with only a limited number of proteins-(actually, many viruses encode only 4 or 5 gene products). Importantly, insects are infected by a wide variety of viruses, some of which are insect-specific, but others also infect humans (arthropod-borne). Recent studies have demonstrated that many human viruses can productively infect *Drosophila* S2 cells including Vesicular Stomatitis Virus, Sindbis Virus, Rift Valley Fever Virus, Dengue virus, West Nile Virus ([51,52] and our unpublished data). The insect-specific viruses, Flock House Virus and *Drosophila* C virus (DCV) have also been extensively studied in fruit flies [51–56]. The first genome-wide RNAi screen for host factors that impact pathogen replication was performed using DCV infection of S2 cells [53]. DCV resembles human picornaviruses such as poliovirus and is a natural pathogen of fruit flies. The screen identified 110 genes that, when lost, block viral replication (Table 1). Two major categories of genes were identified in the screen. First, it was found that attenuation of the translation machinery blocked infection by DCV in *Drosophila* cells and in adult flies, as well as poliovirus replication in human cells. An unrelated virus, without an internal ribosome entry site, was not affected by depletion of the translation machinery. This demonstrates the power of the *Drosophila* system to uncover essential factors in human cells, and that the cross comparisons between different pathogens reveals specificity in the strategies used by different pathogens to exploit and subvert the host. The COPI coatamer was also identified as a cellular machine that is required for the generation of a vesicular compartment necessary for DCV replication as well as poliovirus replication in human cells [54]. Interestingly, the COPI coatamer blocks Vesicular Stomatitis Virus replication (VSV) although this virus does not remodel the membranes within the cell as picornaviruses do (Unpublished data). Therefore, it is likely to be required for a different aspect of VSV replication. The COPI coatamer was also found to be required for phagocytosis, suggesting that some cellular machines may play multiple different roles during infection, depending on the pathogen. Therefore, more mechanistic studies are necessary to assign a function to genes identified in these screens.

Future Directions

The recent development of genome-wide RNAi screening for host factors involved in microbial infection has identified hundreds of cellular genes that impact infection and opened up new avenues of research. It is clear that screening methodologies and technologies are in their infancy, and that these methodologies will continue to improve in efficacy. As the assays become more robust and as the downstream validation is streamlined, using richer more quantitative and statistically robust metrics, we will undoubtedly learn more about the host factors required for infection of particular pathogens. Further validation of candidate genes not only in cultured *Drosophila* cells but in flies and in mammalian systems is becoming simpler and will further our understanding of the biology. This will be facilitated by the availability of genome-wide libraries of transgenic flies that carry inverted repeat constructs against each gene allowing for directed RNAi in vivo in the animal, and the increasing specificity and robustness of siRNA methodologies. Mammalian RNAi screens are also beginning to contribute to our understanding of host-pathogen interactions [57–59], although technical limitations associated with siRNA and shRNA biology still make the *Drosophila* system ideal for RNAi screening. The comparisons between the host factors involved in pathogenesis in these disparate hosts

will also inform the biology. Importantly, the more pathogens that are queried, the deeper our understanding will become of the generalities and specializations used by disparate microbes for infection and pathogenesis and the innate and intrinsic mechanisms used by hosts to inhibit infection.

Acknowledgements

I would like to thank members of the Cherry laboratory as well as S. Ross and N. Silverman for helpful discussions and critical reading of the manuscript. This was supported by NIAID (1R01AI07451, U54 AI 057168) and the Penn Genome Frontiers Institute.

References

1. Rubin GM, Lewis EB. A brief history of *Drosophila*'s contributions to genome research. *Science* 2000;287:2216–2218. [PubMed: 10731135]
2. Tickoo S, Russell S. *Drosophila melanogaster* as a model system for drug discovery and pathway screening. *Curr Opin Pharmacol* 2002;2:555–560. [PubMed: 12324259]
3. Bier E. *Drosophila*, the golden bug, emerges as a tool for human genetics. *Nat Rev Genet* 2005;6:9–23. [PubMed: 15630418]
4. Veraksa A, Del Campo M, McGinnis W. Developmental patterning genes and their conserved functions: from model organisms to humans. *Mol Genet Metab* 2000;69:85–100. [PubMed: 10720435]
5. Cherry S, Silverman N. Host-pathogen interactions in *drosophila*: new tricks from an old friend. *Nat Immunol* 2006;7:911–917. [PubMed: 16924255]
6. Lemaitre B, Hoffmann J. The host defense of *Drosophila melanogaster*. *Annu Rev Immunol* 2007;25:697–743. [PubMed: 17201680]
7. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette *spatzle/Toll/cactus* controls the potent antifungal response in *Drosophila* adults. *Cell* 1996;86:973–983. [PubMed: 8808632]
8. Pinheiro VB, Ellar DJ. How to kill a mocking bug? *Cell Microbiol* 2006;8:545–557. [PubMed: 16548881]
9. Elrod-Erickson M, Mishra S, Schneider D. Interactions between the cellular and humoral immune responses in *Drosophila*. *Curr Biol* 2000;10:781–784. [PubMed: 10898983]
10. Medzhitov R, Janeway C Jr. Innate immune recognition: mechanisms and pathways. *Immunol Rev* 2000;173:89–97. [PubMed: 10719670]
11. Berget SM, Moore C, Sharp PA. Spliced segments at the 5' terminus of adenovirus 2 late mRNA. *Proc Natl Acad Sci U S A* 1977;74:3171–3175. [PubMed: 269380]
12. Payvar F, Wrange O, Carlstedt-Duke J, Okret S, Gustafsson JA, Yamamoto KR. Purified glucocorticoid receptors bind selectively in vitro to a cloned DNA fragment whose transcription is regulated by glucocorticoids in vivo. *Proc Natl Acad Sci U S A* 1981;78:6628–6632. [PubMed: 6273877]
13. Perrimon N, Mathey-Prevot B. Applications of high-throughput RNA interference screens to problems in cell and developmental biology. *Genetics* 2007;175:7–16. [PubMed: 17244760]
14. Ramadan N, Flockhart I, Booker M, Perrimon N, Mathey-Prevot B. Design and implementation of high-throughput RNAi screens in cultured *Drosophila* cells. *Nat Protoc* 2007;2:2245–2264. [PubMed: 17853882] This study systematically discusses the steps involved in developing a high-throughput cell-based screen. Details on experimental design and caveats are important to consider when using data-sets obtained using these methods
15. Ip YT, Reach M, Engstrom Y, Kadalayil L, Cai H, Gonzalez-Crespo S, Tatei K, Levine M. Dif, a dorsal-related gene that mediates an immune response in *Drosophila*. *Cell* 1993;75:753–763. [PubMed: 8242747]
16. Arbouzova NI, Zeidler MP. JAK/STAT signalling in *Drosophila*: insights into conserved regulatory and cellular functions. *Development* 2006;133:2605–2616. [PubMed: 16794031]

17. Agaisse H, Petersen UM, Boutros M, Mathey-Prevot B, Perrimon N. Signaling role of hemocytes in *Drosophila* JAK/STAT-dependent response to septic injury. *Dev Cell* 2003;5:441–450. [PubMed: 12967563]
18. Dostert C, Jouanguy E, Irving P, Troxler L, Galiana-Arnoux D, Hetru C, Hoffmann JA, Imler JL. The Jak-STAT signaling pathway is required but not sufficient for the antiviral response of *Drosophila*. *Nat Immunol* 2005;6:946–953. [PubMed: 16086017]
19. Foley E, O'Farrell PH. Functional dissection of an innate immune response by a genome-wide RNAi screen. *PLoS Biol* 2004;2:E203. [PubMed: 15221030]
20. Kleino A, Valanne S, Ulvila J, Kallio J, Myllymaki H, Enwald H, Stoven S, Poidevin M, Ueda R, Hultmark D, et al. Inhibitor of apoptosis 2 and TAK1-binding protein are components of the *Drosophila* Imd pathway. *EMBO J* 2005;24:3423–3434. [PubMed: 16163390]
21. Gesellchen V, Kutenkeuler D, Steckel M, Pelte N, Boutros M. An RNA interference screen identifies Inhibitor of Apoptosis Protein 2 as a regulator of innate immune signalling in *Drosophila*. *EMBO Rep* 2005;6:979–984. [PubMed: 16170305]
22. Valanne S, Kleino A, Myllymaki H, Vuoristo J, Ramet M. Iap2 is required for a sustained response in the *Drosophila* Imd pathway. *Dev Comp Immunol* 2007;31:991–1001. [PubMed: 17343912]
23. Baeg GH, Zhou R, Perrimon N. Genome-wide RNAi analysis of JAK/STAT signaling components in *Drosophila*. *Genes Dev* 2005;19:1861–1870. [PubMed: 16055650]
24. Muller P, Kutenkeuler D, Gesellchen V, Zeidler MP, Boutros M. Identification of JAK/STAT signalling components by genome-wide RNA interference. *Nature* 2005;436:871–875. [PubMed: 16094372]
25. Stuart LM, Ezekowitz RA. Phagocytosis and comparative innate immunity: learning on the fly. *Nat Rev Immunol* 2008;8:131–141. [PubMed: 18219310]
26. Ramet M, Manfrulli P, Pearson A, Mathey-Prevot B, Ezekowitz RA. Functional genomic analysis of phagocytosis and identification of a *Drosophila* receptor for *E. coli*. *Nature* 2002;416:644–648. [PubMed: 11912489]
27. Kocks C, Cho JH, Nehme N, Ulvila J, Pearson AM, Meister M, Strom C, Conto SL, Hetru C, Stuart LM, et al. Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in *Drosophila*. *Cell* 2005;123:335–346. [PubMed: 16239149] In this study the authors combine microarray data and RNAi to identify a novel phagocytic receptor in flies. This is the first member of a new class of recognition receptors
28. Ramet M, Pearson A, Manfrulli P, Li X, Koziel H, Gobel V, Chung E, Krieger M, Ezekowitz RA. *Drosophila* scavenger receptor CI is a pattern recognition receptor for bacteria. *Immunity* 2001;15:1027–1038. [PubMed: 11754822]
29. Kurucz E, Markus R, Zsomboki J, Folkl-Medzihradsky K, Darula Z, Vilmos P, Udvardy A, Krausz I, Lukacsovich T, Gateff E, et al. Nimrod, a putative phagocytosis receptor with EGF repeats in *Drosophila* plasmatocytes. *Curr Biol* 2007;17:649–654. [PubMed: 17363253]
30. Netea MG, Brown GD, Kullberg BJ, Gow NA. An integrated model of the recognition of *Candida albicans* by the innate immune system. *Nat Rev Microbiol* 2008;6:67–78. [PubMed: 18079743]
31. Stroschein-Stevenson SL, Foley E, O'Farrell PH, Johnson AD. Identification of *Drosophila* gene products required for phagocytosis of *Candida albicans*. *PLoS Biol* 2006;4:e4. [PubMed: 16336044] How fungi are recognized and taken up by cells is not well understood. This visual screen reveals a large number of genes that impact phagocytosis of *C. albicans*
32. Philips JA, Rubin EJ, Perrimon N. *Drosophila* RNAi screen reveals CD36 family member required for mycobacterial infection. *Science* 2005;309:1251–1253. [PubMed: 16020694] Mycobacterial species are important human pathogens. Philips et al used a model mycobacteria to elucidate the cellular factors required both for phagocytosis and intracellular replication
33. Agaisse H, Burrack LS, Philips JA, Rubin EJ, Perrimon N, Higgins DE. Genome-wide RNAi screen for host factors required for intracellular bacterial infection. *Science* 2005;309:1248–1251. [PubMed: 16020693] A screen for cellular factors that impact *Listeria* infection revealed a large number of genes that when lost inhibit infection. Amongst the genes are factors that impact a variety of steps in the intracellular lifecycle
34. Pielage JF, Powell KR, Kalman D, Engel JN. RNAi Screen Reveals an Abl Kinase-Dependent Host Cell Pathway Involved in *Pseudomonas aeruginosa* Internalization. *PLoS Pathog* 2008;4:e1000031.

[PubMed: 18369477] Signaling pathways clearly play an important role in uptake. This study reveals an important role for Abl kinase signaling in disparate hosts

- 35•. Cheng LW, Viala JP, Stuurman N, Wiedemann U, Vale RD, Portnoy DA. Use of RNA interference in *Drosophila* S2 cells to identify host pathways controlling compartmentalization of an intracellular pathogen. *Proc Natl Acad Sci U S A* 2005;102:13646–13651. [PubMed: 16157870] This study uses a variety of genetically modified *Listeria* to systematically identify host factors required for different steps in the bacterial infection cycle
- 36•. Stuart LM, Boulais J, Charriere GM, Hennessy EJ, Brunet S, Jutras I, Goyette G, Rondeau C, Letarte S, Huang H, et al. A systems biology analysis of the *Drosophila* phagosome. *Nature* 2007;445:95–101. [PubMed: 17151602] An elegant combination of proteomics and functional genomics identified a large number of essential components of the phagosome in *Drosophila*
37. Antony B. Membrane deformation by protein coats. *Curr Opin Cell Biol* 2006;18:386–394. [PubMed: 16782321]
38. TerBush DR, Maurice T, Roth D, Novick P. The Exocyst is a multiprotein complex required for exocytosis in *Saccharomyces cerevisiae*. *EMBO J* 1996;15:6483–6494. [PubMed: 8978675]
- 39•. Derre I, Pypaert M, Dautry-Varsat A, Agaisse H. RNAi screen in *Drosophila* cells reveals the involvement of the Tom complex in *Chlamydia* infection. *PLoS Pathog* 2007;3:1446–1458. [PubMed: 17967059] Using a model *Chlamydia* species this study identified a number of genes including the mitochondrial Tom complex as essential for *C. caviae* infection
40. Bhavsar AP, Guttman JA, Finlay BB. Manipulation of host-cell pathways by bacterial pathogens. *Nature* 2007;449:827–834. [PubMed: 17943119]
41. Sundaramurthy V, Pieters J. Interactions of pathogenic mycobacteria with host macrophages. *Microbes Infect* 2007;9:1671–1679. [PubMed: 18023233]
42. Dionne MS, Ghorri N, Schneider DS. *Drosophila melanogaster* is a genetically tractable model host for *Mycobacterium marinum*. *Infect Immun* 2003;71:3540–3550. [PubMed: 12761139]
- 43•. Koo IC, Ohol YM, Wu P, Morisaki JH, Cox JS, Brown EJ. Role for lysosomal enzyme beta-hexosaminidase in the control of mycobacteria infection. *Proc Natl Acad Sci U S A* 2008;105:710–715. [PubMed: 18180457] This study identified a novel metabolic enzyme required for mycobacterial infection of mammalian cells
44. Philips JA, Porto MC, Wang H, Rubin EJ, Perrimon N. ESCRT factors restrict mycobacterial growth. *Proc Natl Acad Sci U S A*. 2008
45. Jules M, Buchrieser C. *Legionella pneumophila* adaptation to intracellular life and the host response: clues from genomics and transcriptomics. *FEBS Lett* 2007;581:2829–2838. [PubMed: 17531986]
- 46•. Dorer MS, Kirton D, Bader JS, Isberg RR. RNA interference analysis of *Legionella* in *Drosophila* cells: exploitation of early secretory apparatus dynamics. *PLoS Pathog* 2006;2:e34. [PubMed: 16652170] This focused study took advantage of the ease of performing binary RNAi to identify partially redundant pathways required for *Legionella* vacuole production
47. Gupta RS, Griffiths E. *Chlamydiae*-specific proteins and indels: novel tools for studies. *Trends Microbiol* 2006;14:527–535. [PubMed: 17049238]
48. Elwell C, Engel JN. *Drosophila melanogaster* S2 cells: a model system to study *Chlamydia* interaction with host cells. *Cell Microbiol* 2005;7:725–739. [PubMed: 15839901]
- 49•. Elwell CA, Ceesay A, Kim JH, Kalman D, Engel JN. RNA Interference Screen Identifies Abl Kinase and PDGFR Signaling in *Chlamydia trachomatis* Entry. *PLoS Pathog* 2008;4:e1000021. [PubMed: 18369471] This study took advantage of the fact that the human pathogen *C. trachomatis* infects *Drosophila* cells, to screen for cellular factors that impact early steps in the bacterial infection cycle to identify signaling pathways essential for infection in mammals
50. Neupert W, Herrmann JM. Translocation of proteins into mitochondria. *Annu Rev Biochem* 2007;76:723–749. [PubMed: 17263664]
51. Cherry S, Perrimon N. Entry is a rate-limiting step for viral infection in a *Drosophila melanogaster* model of pathogenesis. *Nat Immunol* 2004;5:81–87. [PubMed: 14691479]
52. Galiana-Arnoux D, Dostert C, Schneemann A, Hoffmann JA, Imler JL. Essential function in vivo for Dicer-2 in host defense against RNA viruses in *Drosophila*. *Nat Immunol* 2006;7:590–597. [PubMed: 16554838]

- 53•. Cherry S, Doukas T, Armknecht S, Whelan S, Wang H, Sarnow P, Perrimon N. Genome-wide RNAi screen reveals a specific sensitivity of IRES-containing RNA viruses to host translation inhibition. *Genes Dev* 2005;19:445–452. [PubMed: 15713840] This was the first genome-wide RNAi screen performed screening for host-pathogen interactions. The authors identified a specific requirement for high levels of the translation machinery for picornaviruses both in insects and mammals
54. Cherry S, Kunte A, Wang H, Coyne C, Rawson RB, Perrimon N. COPI activity coupled with fatty acid biosynthesis is required for viral replication. *PLoS Pathog* 2006;2:e102. [PubMed: 17040126]
55. van Rij RP, Saleh MC, Berry B, Foo C, Houk A, Antoniewski C, Andino R. The RNA silencing endonuclease Argonaute 2 mediates specific antiviral immunity in *Drosophila melanogaster*. *Genes Dev* 2006;20:2985–2995. [PubMed: 17079687]
56. Wang XH, Aliyari R, Li WX, Li HW, Kim K, Carthew R, Atkinson P, Ding SW. RNA interference directs innate immunity against viruses in adult *Drosophila*. *Science* 2006;312:452–454. [PubMed: 16556799]
- 57•. Brass AL, Dykxhoorn DM, Benita Y, Yan N, Engelman A, Xavier RJ, Lieberman J, Elledge SJ. Identification of host proteins required for HIV infection through a functional genomic screen. *Science* 2008;319:921–926. [PubMed: 18187620] This was the first genome-wide siRNA screen for host factors required for viral replication in human cells. The authors identified a large number of genes involved in a wide variety of cellular processes
- 58•. Pelkmans L, Fava E, Grabner H, Hannus M, Habermann B, Krausz E, Zerial M. Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis. *Nature* 2005;436:78–86. [PubMed: 15889048] This was the first study to use a genome-scale library to study virus entry. This comparative analysis revealed that there are clear differences in host factor requirements for viruses known to enter cells by disparate routes
59. Randall G, Panis M, Cooper JD, Tellinghuisen TL, Sukhodolets KE, Pfeffer S, Landthaler M, Landgraf P, Kan S, Lindenbach BD, et al. Cellular cofactors affecting hepatitis C virus infection and replication. *Proc Natl Acad Sci U S A* 2007;104:12884–12889. [PubMed: 17616579]

Figure 1. RNAi screening workflow. This flow chart describes the critical decisions and optimizations that must be considered when developing and reading high-throughput cell-based RNAi screens.

Host-Pathogen screens in *Drosophila* cells. This table describes the technical parameters of the screens described including: screen type, cell type, library used, reporter used, read-out, analysis and genes identified.

TABLE 1	Screen	Reference	Cell Line	Library	Inducer	Reporter	Mode	Analysis	Positive Regulators	Negative Regulators
Signaling	Imd Signaling	Foley et al	S2	7,216 conserved genes	LPS	diptericin-beta-glactosidase	microscope	visual	49	72
	Imd Signaling	Kleino et al	S2	6,700 hemocyte expressed ESTs	heat killed <i>E. Coli</i>	attacin-luciferase vs. control	plate-reader	statistical	4	nd
	JAK/Stat Signaling	Muller et al.	Kc176	genome-wide library	Upd Overexpression	Draf 10X STAT sites-luciferase vs. control	plate-reader	statistical	67	24
Phagocytosis	JAK/Stat Signaling	Baeg et al.	S2	genome-wide library	Endogenous Activity	SOCS36E 10X STAT sites-luciferase vs. control	plate-reader	statistical	29	92
	General Phagocytosis	Ramet et al.	S2	1,000 dsRNAs S2expressed library		GFP- <i>E. coli</i> , GFP- <i>S. aureus</i>	FACS	Fold	34	nd
	Serpent-dependent	Kocks, et al.	S2	45 differentially expressed genes		GFP- <i>E. coli</i> , GFP- <i>S. aureus</i>	FACS	Fold	1	nd
Live Bacterial	<i>Candida albicans</i>	Stroschein-Stevenson, et al.	S2	7,216 conserved genes		GFP- <i>C. albicans/anti-C. albicans</i>	microscope	visual	184	nd
	<i>Mycobacterium Fortuitum</i>	Philips, et al.	S2	genome-wide library		Induced GFP- <i>M. fortuitum</i>	microscope	statistical	85	nd
	<i>Mycobacterium Marinum</i>	Koo, et al.	S2	1000 genes		GFP- <i>M. marinum</i>	FACS	fold	nd	2
	<i>Listeria Monocytogenes</i>	Agaisse, et al.	S2	genome-wide library		GFP- <i>L. monocytogenes</i>	microscope	visual	167*	48
	<i>Listeria Monocytogenes</i>	Cheng, et al.	S2	7,216 conserved genes	(wt, LLO-minus, LLO-toxic)	anti- <i>Listeria</i>	microscope	visual	89,29,8	nd
	<i>Legionella pneumophila</i>	Dorer, et al.	Kc167	10dsRNAs 73dsRN As trafficking		GFP- <i>L. pneumophila</i>	microscope	visual	12 singles, new combos	nd
Virus	<i>Chlamydia caviae</i>	Derre, et al	S2	genome-wide library		anti- <i>C. caviae</i>	microscope	visual	54	nd
	<i>Drosophila C virus</i>	Cherry, et al.	S2	genome-wide library		anti- <i>Drosophila C virus</i>	microscope	visual	110	nd