

Genome-Wide RNAi Screen for Host Factors Required for Intracellular Bacterial Infection

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Most studies of host-pathogen interactions have focused on pathogen-specific virulence determinants. Here, we report a genome-wide RNA interference screen to identify host factors required for intracellular bacterial pathogenesis. Using *Listeria monocytogenes*, a cytosolic pathogen, and *Drosophila* cells, 305 dsRNAs targeting a wide range of cellular functions were identified that altered *L. monocytogenes* infection. Comparison to a similar screen with *Mycobacterium fortuitum*, a vacuolar pathogen, identified host factors that may play a general role in intracellular pathogenesis and factors that specifically affect access to the cytosol by *L. monocytogenes*.

During bacterial infections, macrophages play a critical role in eliminating engulfed pathogens. However, intracellular bacterial pathogens have evolved varying strategies to avoid elimination by host macrophages (1). One strategy used by pathogens, such as *Mycobacterium tuberculosis*, is to modify the phagosomal compartment to allow for vacuolar replication (2). Other bacterial pathogens, such as *Listeria monocytogenes*, escape the phagocytic vacuole to enter the host cell cytosol where replication occurs (3). While numerous bacterial determinants that facilitate intracellular infection have been characterized from diverse bacterial species (4), less is known about the host factors that are exploited or subverted by intracellular bacterial pathogens. Here, we report the results of a genome-wide RNA interference (RNAi) screen conducted in *Drosophila* SL2 cells to identify host factors required for infection by *L. monocytogenes*, a cytosolic pathogen. In addition, we present the results of a comparison to a similar RNAi screen conducted with *Mycobacterium fortuitum*, a vacuolar pathogen (5).

Both *Drosophila* and cultured *Drosophila* cells are tractable models for analysis of *L. monocytogenes* pathogenesis (6, 7). We tested the ability of macrophage-like *Drosophila* SL2 cells to support intracellular infection by *L. monocytogenes*. DH-L1039, a GFP-expressing *L. monocytogenes* strain derived from wild-type 10403S, replicated within *Drosophila* SL2 cells (fig. S1, A and B). In contrast, GFP fluorescence of DH-L1137, a variant lacking the pore-forming cytolysin listeriolysin O (LLO), was punctate in appearance and growth was inefficient in SL2 cells (fig. S1, A and B), consistent with LLO-negative bacteria remaining trapped within phagocytic vacuoles (3, 8). Next, we developed a microscopy-based, high-throughput RNAi screen to identify host factors required for intracellular infection by *L. monocytogenes* (Fig. 1A) (9). We confirmed the feasibility of the approach by analyzing the impact of β -COPI dsRNA treatment on *L. monocytogenes* infection. Interfering with β -COPI expression by RNAi results in a strong defect in *E. coli* phagocytosis (10). The impact of blocking *L. monocytogenes* entry by β -COPI dsRNA treatment was clearly detectable by fluorescence microscopy (fig. S1C). For the screen, we used a library of ~21,300 dsRNAs (11) that target >95% of annotated genes in the *Drosophila* genome (12) and identified several phenotypes (Fig. 1, B to F): (i) Decreased GFP fluorescence (Down phenotype). In some instances only a few host cells were infected, but in those cells the infection was as robust as observed in control wells, consistent with a defect in entry (Fig. 1C). In other cases, the percentage of host cells displaying GFP fluorescence appeared to be similar to control wells, yet the intensity of GFP fluorescence per cell was decreased, consistent with a defect in intracellular replication (Fig. 1D). Combinations of these two phenotypes were also observed. (ii) Punctate GFP fluorescence (Spots phenotype).

This phenotype was similar to the phenotype observed during infection by DH-L1137 (fig. S1A) and is consistent with a defect in vacuole escape. However, bacteria in dsRNA treated wells appeared to be capable of increased replication within the localized clusters (Fig. 1E). (iii) Increased GFP fluorescence (Up phenotype). The intensity of GFP fluorescence per infected SL2 cell was more robust, consistent with an increase in intracellular replication rate or increased bacterial uptake (Fig. 1F).

From the primary RNAi screen, we identified 358 *Drosophila* genes that potentially affect *L. monocytogenes* infection, including genes coding for ribosome components (61 genes) and proteasome components (25 genes). With the exception of the dsRNAs targeting ribosome and proteasome components (13), the candidate dsRNAs identified in the primary RNAi screen were re-tested at least six times and ~70% were confirmed as affecting *L. monocytogenes* infection. Of these dsRNAs, less than 13% were shown to have effects on host cell viability in a previous screen using the same dsRNA library (11), suggesting a specific effect on intracellular bacterial infection rather than non-specific effects on the host cells. The putative host factors required for *L. monocytogenes* infection spanned a wide range of cellular functions (tables S1 and S2). Many of the identified targets resulting in a Down phenotype are predicted to be involved in vesicular trafficking (19%), signal transduction (10%), and cytoskeletal organization (10%). Knockdown of many of these targets resulted in a phenotype consistent with an entry defect, including Syx5 (vesicular trafficking) (Fig. 1C), Cdc42 (signal transduction) and Arp2/3 complex members (cytoskeleton). Other targets in these categories had phenotypes consistent with defects in steps beyond entry, such as Tor (target of rapamycin) (signal transduction) (Fig. 1D) and Rab2 (vesicular trafficking) (13). Furthermore, dsRNAs targeting 48 *Drosophila* genes caused an Up phenotype (Fig. 2A and table S1). The gene products of these targets are predominantly predicted to be involved in cell cycle (40%) and RNA processing (13%).

To determine if the identified host factors were specific to *L. monocytogenes* infection or more generally required for intracellular pathogenesis, we compared the results to a similar RNAi-based screen with *M. fortuitum* (5, 9). We focused our comparative analysis on candidates that caused decreased infection (Fig. 2, B and C). The majority of dsRNAs that decreased infection by both pathogens target factors predicted to be involved in vesicular trafficking or cytoskeleton components (table S3 and Fig. 2C). Many of these may be required for non-specific uptake of both pathogens, because they were also found to affect phagocytosis of *E. coli* (5). However, some host factors appeared to be specific for entry. For example, knockdown of CG7228 (Pes), a member of the CD36 family of scavenger

receptors, reduced entry of both *L. monocytogenes* (fig. S2) and *M. fortuitum*, but had no effect on uptake of *E. coli* or *S. aureus* (5). In addition, several candidates affecting both pathogens appeared to have roles other than entry (13).

We also identified dsRNAs that decreased infection by one, but not the other pathogen. For example, dsRNA targeting CG6121 decreased intracellular infection by *M. fortuitum*, but did not interfere with *L. monocytogenes* infection (Fig. 3). CG6121 (dTip60) is a member of a complex with histone acetyltransferase activity (14). Two other members of the *Drosophila* Tip60 complex, dom and E(Pc), were also identified as affecting *M. fortuitum* infection. HIV Tat protein interaction with Tip60 in mammalian cells alters expression of host cell genes (15). Thus, it is possible that *M. fortuitum* similarly targets the Tip60 complex to modify the expression of specific host factors. In all, 17 dsRNAs that decreased infection by *M. fortuitum* did not affect *L. monocytogenes* infection. Six dsRNAs that decreased infection by *M. fortuitum* caused an Up phenotype for *L. monocytogenes* (table S3). A total of 59 dsRNAs, including those targeting a putative glucose transporter, CG10960, decreased infection by *L. monocytogenes*, but did not affect *M. fortuitum* (table S3 and Fig. 3). Ten dsRNAs that caused a Down phenotype for *L. monocytogenes* appeared to increase *M. fortuitum* intracellular growth (table S3). Furthermore, all 11 dsRNAs resulting in the Spots phenotype for *L. monocytogenes* (table S1) did not detectably affect *M. fortuitum* infection. As confirmed by electron microscopy, the Spots phenotype corresponded to a vacuolar escape defect. In untreated SL2 cells, *L. monocytogenes* were found free in the cytosol (Fig. 3C), while treatment with CG11814 dsRNA, which caused a Spots phenotype, resulted in membrane-bound compartments harboring multiple bacteria (Fig. 3D). CG11814 is predicted to have roles in lysosomal transport. Spots vacuoles appeared to be more permissive for growth of *L. monocytogenes*. Indeed, LLO-negative bacteria replicated to a 2-fold greater extent in SL2 cells treated with CG11814 dsRNA compared to untreated SL2 cells (fig. S3). 52% of membrane-bound compartments containing one or more bacteria in CG11814 dsRNA-treated cells were surrounded by multilamellar structures (Fig. 3E) compared to 8% in untreated control cells. Similar structures surround mutants of another cytosolic pathogen, *Shigella flexneri* (16). These multilamellar structures result from an attempt of the host cell to control intracellular infection by a process related to autophagy (16). Thus, autophagy may represent a defense mechanism that limits *L. monocytogenes* infection (13). Because infection of *Drosophila* cells by intracellular bacterial pathogens is similar to infection of mammalian host cells, it is likely that many homologous mammalian host factors will be conserved in their requirement for intracellular infection.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/11116008/DC1

Materials and Methods

SOM Text

Figs. S1 to S4

Tables S1 to S4

References

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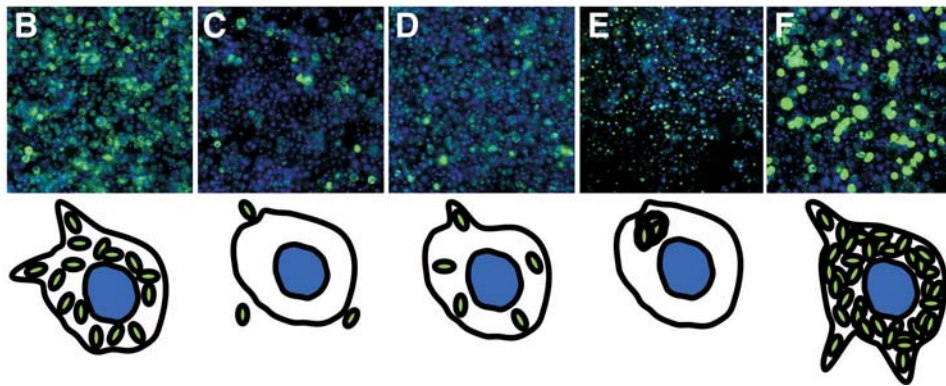
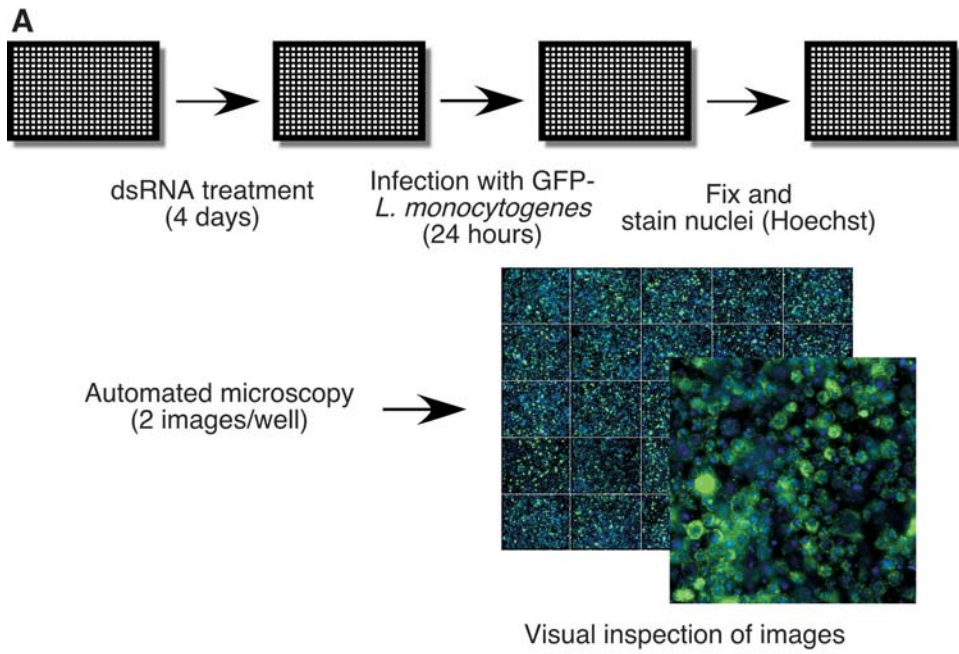
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Fig. 1. RNAi screening procedure and phenotypes observed.

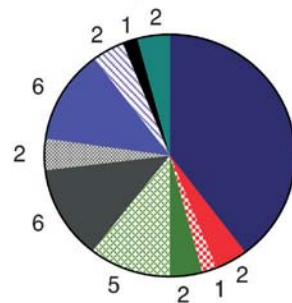
(A) Screen design. (B-F) Candidates representative of the observed phenotypes. (B) No dsRNA: (control). (C) Syx5: Down phenotype, “decreased percent”. (D) Tor: Down phenotype “decreased per cell”. (E) CG5691: Spots phenotype. (F) CG3605: Up phenotype. Below each image is a schematic depiction of the corresponding phenotype.

Fig. 2. Functional categories of dsRNA targets identified in *L. monocytogenes* and *M. fortuitum* RNAi screens. [(A) to (C)] Functional categories identified. The numbers surrounding the charts indicate the number of target genes within each functional category. Asterisks (*) indicate targets other than proteasome or ribosome subunits that are categorized as proteolysis or translation. (A) dsRNAs resulting in an Up phenotype for *L. monocytogenes*. (B) dsRNAs resulting in a Down phenotype for *L. monocytogenes*. (C) dsRNAs resulting in a Down phenotype for both *L. monocytogenes* and *M. fortuitum*.

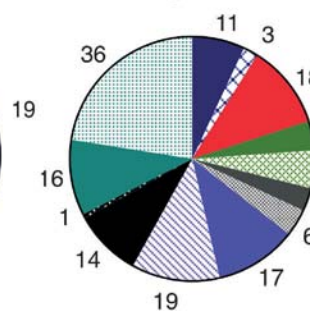
Fig. 3. Representative phenotypes for dsRNAs that uniquely affect *L. monocytogenes* or *M. fortuitum* infection. (A and B) SL2 cells were untreated (top), treated with CG6121 dsRNA (middle) or treated with CG10960 dsRNA (bottom) and infected with *L. monocytogenes* (A) or *M. fortuitum* (B) expressing GFP (green) (5). At 24 hours post-infection (*L. monocytogenes*) or 48 hours post-infection (*M. fortuitum*) cells were fixed and stained with Hoechst dye (blue). Fluorescent images (20X) are representative of infections performed at least six times for each pathogen. (C to E) Electron micrographs of (C) untreated or [(D) and (E)] CG11814 dsRNA-treated SL2 cells infected with wild-type *L. monocytogenes*. Arrows indicate membranes surrounding bacteria in the CG11814 dsRNA-treated cells. Scale bars represent 0.5 μ m.



A 48 targets



B 160 targets



C 91 targets

