Drosophila RNAi Screen Reveals CD36 Family Member Required for Mycobacterial Infection

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Certain pathogens, such as Mycobacterium tuberculosis, survive within the hostile intracellular environment of a macrophage. To identify host factors required for mycobacterial entry and survival within macrophages, we performed a genomewide RNAi screen in Drosophila macrophage-like cells, using Mycobacterium fortuitum. We identified factors required for general phagocytosis, as well as those needed specifically for mycobacterial infection. One specific factor, Peste (Pes), is a CD36 family member required for uptake of mycobacteria, but not E. coli or S. aureus. Moreover, mammalian class B scavenger receptors (SRs) conferred uptake of bacteria to nonphagocytic cells, with SR-BI and SR-BII uniquely mediating uptake of M. fortuitum, which suggests a conserved role for class B SRs in pattern recognition and innate immunity.

About one-third of the world’s population is infected by M. tuberculosis, and it is responsible for more deaths yearly than any other bacterial pathogen. In addition, other pathogenic mycobacteria, including M. fortuitum, are capable of causing infection in humans (1). While macrophages play a central role in host defense, recognizing and destroying pathogens, pathogenic mycobacteria are able to survive within this hostile environment. Mycobacteria can escape phagosome-lysosome fusion (2) and grow in a variety of evolutionarily divergent phagocytic cells, including mammalian macrophages, fish monocytes (3), fly hemocytes (4), and amoeba (5). Thus, mycobacteria appear to target evolutionarily conserved molecules for intracellular survival and growth. Although several factors involved in phagosome maturation arrest have been studied (6), there has been no systematic, genetic approach for identifying host factors required for mycobacterial survival. Here we describe a model of infection using Drosophila S2 cells, a macrophage-like cell line (7–9) that is readily amenable to RNA interference (RNAi). This allowed us to conduct a systematic functional genomic screen to identify host factors required for uptake and growth of mycobacteria.

M. fortuitum has several properties making it a useful model mycobacterium. Like M. tuberculosis, it restricts interferon-gamma-induced nitric oxide production and limits phagosome fusion with lysosomes (10), suggesting common virulence properties with other mycobacteria. In addition, M. fortuitum infects Diptera in nature (11) so flies may have innate mechanisms to combat infection. Practically, M. fortuitum grows relatively rapidly at 25°C, the temperature at which S2 cells grow, thus facilitating development of a robust assay of intracellular growth. To detect intracellular growth, we tested constructs in which green fluorescent protein (GFP) expression is under control of the map24 and map49 promoters that are induced when the fish pathogen Mycobacterium marinum infects macrophages (12). We found that these promoters could also be used to efficiently detect intracellular growth of M. fortuitum (fig. S1). By 24 h post-infection of S2 cells, expression of map24 and map49 was induced (figs. S1 and S2; and Fig. 1A).

In mammalian cells, recruitment of the Arp2/3 complex is required for phagocytosis (13), while expression of a dominant negative version of Rab5 causes internalized Mycobacterium avium to be delivered to the lysosome (14). Thus, we reasoned that dsRNAs targeting Arp14D and Rab5 would decrease infection in Drosophila cells. Indeed, we found that these dsRNAs blocked intracellular growth of M. fortuitum (Fig. 1A). Using this assay, we performed a genomewide RNAi screen in which each unique dsRNA was tested for its ability to disrupt infection based upon visual inspection and automated image analysis (15) (Fig. 1B). We found 6/7 Arp2/3 components, 5/7 COPI components, and all six alleles of actin, suggesting a false negative rate of less than 20%. Upon repeat testing of the candidates identified in the primary screen, 86 dsRNAs decreased infection by 2 S.D. (P < 0.05; tables S1 and S3). These dsRNAs target genes involved in basic cellular processes, the largest categories of which are predicted to have a role in vesicle trafficking and actin cytoskeletal organization (Fig. 1C).
Because GFP expression requires internalization of bacteria, we expected to find dsRNAs that block general phagocytosis. Indeed, we found that 54 dsRNAs caused a significant decrease in phagocytosis as measured by the uptake of fluorescent Escherichia coli (P < 0.01; table S2 and fig. S3). Approximately two-thirds of these dsRNAs target factors involved in the actin cytoskeleton and vesicle trafficking (Fig. 1C). Nearly all of the actin cytoskeletal components that affected M. fortuitum infection appeared to be generally required for phagocytosis, including Cdc42, the Arp2/3 complex, actin capping proteins, and coflin, all molecules previously implicated in phagocytosis (13). The majority of vesicle trafficking genes also affected phagocytosis, with exceptions such as CG1515 (predicted to have SNAP receptor activity) and Rab2.

However, the requirement for many genes for infection could not be explained by their role in phagocytosis. For example, dsRNA that targets chickadee (chic), which encodes a Profilin, caused a small increase in phagocytosis (118% of GFP treated well; P < 0.05), similar to what has been reported in hemocytes heterozygous for chic (chic^{01320/+}; 9). Hence, Chic is unlikely to act at the level of uptake and may play a unique role in mycobacterial infection. Some dsRNAs, such as those that target Rab5 and Rac2, caused a mild defect in phagocytosis relative to their severe defect in the M. fortuitum infection, indicating that they may have additional roles later in infection. Finally, there were categories of host factors that were not required for bacterial uptake in general but were nonetheless needed for infection with M. fortuitum. These include components of the vacuolar ATPase and chromatin factors, along with many that do not fit into distinct categories (Fig. 2C).

We further characterized one host factor that appeared to be specifically required for mycobacterial uptake. The dsRNA that targeted CG7228, a member of the CD36 family of scavenger receptors (SR), blocked infection by M. fortuitum (Fig. 2A), but appeared to be dispensable for phagocytosis in general. dSR-CI, a class C scavenger receptor, makes a small contribution toward the uptake of E. coli and Staphylococcus aureus in S2 cells (8), but we could not detect a similar contribution from CG7228 (Fig. 2B). However, CG7228 was required for uptake of M. smegmatis, as were more general phagocytosis factors (Fig. 2C). In addition, CG7228 is required for uptake of L. monocytogenes (16). The fact that CG7228 is required for infection with M. fortuitum and for uptake of M. smegmatis and L. monocytogenes, whereas it makes no contribution toward the uptake of E. coli or S. aureus, suggests that it functions in pattern recognition, detecting some component of Mycobacteria and Listeria. Based upon its role in bacterial infection, we have named it peste (pes).

To determine whether Pes was sufficient to confer uptake of mycobacteria, we expressed it in human embryonic kidney 293 (HEK293) cells that are refractory to infection with M. fortuitum. HEK293 cells transfected with Pes could be infected with M. fortuitum (Fig. 3A), showing that the Drosophila SR can mediate uptake of mycobacteria in human cells. Pes also caused a small increase in the uptake of E. coli and S. aureus when transfected in HEK293 cells (Fig. 3B and 3C), although it did not seem to be required for their uptake in S2 cells. This apparent discrepancy may be explained by genetic redundancy provided by PGRP-Lc (7), dSR-CI (8), and potentially other SRs that mediate uptake of E. coli and S. aureus in S2 cells.

While in Drosophila there are more than ten class B SRs, in humans and mice there are four-- CD36, SR-BI, its splice isoform SR-BII, and LIMP-II. We tested the ability of these molecules to mediate infection with M. fortuitum and found that human SR-BI and SR-BII (17) resulted in an even greater level of infection than Pes when transfected in HEK293 cells. In contrast, expression of murine CD36 caused little, if any, increase in infection with M. fortuitum (Fig. 3A). However, cells transfected with CD36 were capable of taking up E. coli, and to a lesser extent S. aureus. SR-BI and SR-BII seemed more indiscriminate in terms of their bacterial recognition, as they were able to mediate uptake of all bacteria tested (Fig. 3B and 3C). Whereas SR-BI and SR-BII have been extensively studied for their role in cholesterol transport in liver and steroidogenic tissue, their role in macrophages is less clear (18). We suggest that these receptors may be important in host defense against a broad range of bacteria, including M. fortuitum, E. coli, and S. aureus, and could play a role in the non-opsonic uptake of M. tuberculosis.

Consistent with this idea, fucoidin, a non-specific inhibitor that blocks both class A SRs and SR-BI (19), interferes with binding and uptake of M. tuberculosis in monocyte-derived macrophages (20). CD36 may play a partially overlapping role recognizing bacteria, but is unable to mediate uptake of M. fortuitum. As CD36 augments TLR2 signaling in response to a subset of TLR2 ligands (21), we speculate that CD36, SR-BI, and SR-BII serve partially overlapping roles in both bacterial uptake and TLR signaling, which may represent an evolutionarily conserved strategy in host defense, linking phagocytosis to antimicrobial signaling.

References and Notes
Fig. 1. Host factors required for M. fortuitum infection identified by RNAi. After treatment with dsRNAs, S2 cells were infected with M. fortuitum map24::GFP. dsRNA targeting Arp14D or Rab5 decreased infection compared to controls treated with dsRNA targeting GFP (A). This assay was used as the basis of a genome-wide screen described in Methods (B). A composite image of 35 wells is shown. Pie charts based upon GeneOntology (GO) index biological function (C) show the categories of host factors that reproducibly decreased infection after 3d of dsRNA treatment, as well as the subsets that are required for phagocytosis and those that are apparently phagocytosis independent.

Fig. 2. CG7228 is required in S2 cells for infection by M. fortuitum and uptake of M. smegmatis but not E. coli or S. aureus. (A) dsRNA against CG7228 blocked infection by M. fortuitum compared to controls treated with dsRNA against GFP. (B) uptake of FITC-conjugated E. coli (black bars) and S. aureus (grey bars) is blocked by dsRNA toward dSR-CI, Arp14D, Cdc42, and CG3523, but not CG7228. Data show the percent uptake as compared to untreated cells (see fig. S3). The average of six experiments in which each dsRNA was tested in triplicate is shown with error bars denoting S.D. (*P < 0.05 compared to GFP treated wells based upon a Students t-test.) (C) Antibiotic protection experiments demonstrate the role of CG7228, CG3523, and Cdc42 in the uptake of M. smegmatis. Data are normalized to GFP; error bars indicate S.D. from a representative experiment performed in triplicate.

Fig. 3. Class B SRs mediate uptake of M. fortuitum, E. coli, and S. aureus. HEK293 cells transfected with vector (pcDNA3.1) or expression constructs for CG7228 (Pes), CD36, SR-BI, or SR-BII were (A) infected with M. fortuitum map24::GFP or incubated with (B) FITC-E. coli or (C) FITC-S. aureus. In (A) green represents GFP expression from the map24 promoter, whereas in (B) and (C) green shows FITC signal from internalized bacteria. Nuclei are red. Images are 40X.