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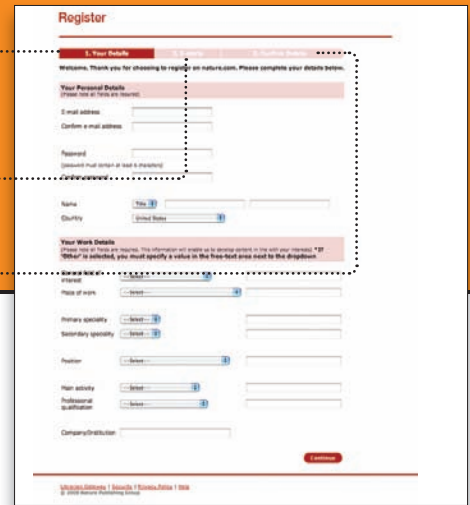
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FIGURE 1. RNAi screen and bioinformatics.

From the Research Article: RNA interference screen for human genes associated with West Nile virus infection. Brittan, M. A., et al. *Nature* 455, 242-245 (11 September 2008) | doi:10.1038/nature07207

The figure shows a flowchart of the RNAi screen strategy and a pie chart of bioinformatics classification. The flowchart details the process from library screening to validation and functional analysis. The pie chart shows the distribution of hits across various biological processes and molecular functions.

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RNA interference screen for human genes associated with West Nile virus infection

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West Nile virus (WNV), and related flaviviruses such as tick-borne encephalitis, Japanese encephalitis, yellow fever and dengue viruses, constitute a significant global human health problem¹. However, our understanding of the molecular interaction of such flaviviruses with mammalian host cells is limited². WNV encodes only 10 proteins, implying that it may use many cellular proteins for infection³. WNV enters the cytoplasm through pH-dependent endocytosis, undergoes cycles of translation and replication, assembles progeny virions in association with endoplasmic reticulum, and exits along the secretory pathway^{4,5,6}. RNA interference (RNAi) presents a powerful forward genetics approach to dissect virus-host cell interactions^{7,8,9,10}. Here we report the identification of 305 host proteins that affect WNV infection, using a human-genome-wide RNAi screen. Functional clustering of the genes revealed a complex dependence of this virus on host cell physiology, requiring a wide variety of molecules and cellular pathways for successful infection. We further demonstrate a requirement for the ubiquitin ligase CBL1 in WNV internalization, a post-entry role for the endoplasmic-reticulum-associated degradation pathway in viral infection, and the monocarboxylic acid transporter MCT4 as a viral replication resistance factor. By extending this study to dengue virus, we show that flaviviruses have both overlapping and unique interaction strategies with host cells. This study provides a comprehensive molecular portrait of WNV-human cell interactions that forms a model for understanding single plus-stranded RNA virus infection, and reveals potential antiviral targets.

The host proteins previously reported to facilitate WNV infection (termed host susceptibility factors; HSFs) comprise endosomal transport regulators and vATPase (for entry), eEF1A, TIA-1/TIAR and HMGCR (for replication), and c-Yes (for secretion)^{7,8,9,10}. Other host proteins may reduce WNV infection (termed host resistance factors, HRFs): components of the antiviral IRF3 pathway are known HRFs of WNV infection¹¹. In this context, we performed a genome-scale small interfering RNA (siRNA)-based screen silencing 21,121 human genes in HeLa cells to comprehensively identify the cellular proteins associated with the early stages of WNV infection, from viral entry through to the intracellular translation of viral RNA. Defects in the later stages of infection, such as replication, assembly or secretion, were not scored by the assay. The assay involved infection of gene-silenced cells with WNV for 24 h, followed by a microscopy-based quantification of the cells immunostained for viral envelope protein to select the candidate host proteins. The screen was done in two steps: a primary screen using a pool of four siRNAs per gene, followed by a validation screen, testing each individual siRNA within the pool separately (for the hits selected in the primary screen) to minimize potential off-target hits (Fig. 1a). The details of the assay and screen are described in Methods and Supplementary Fig. 1.

Figure 1: RNAi screen and bioinformatics. a, West Nile virus RNAi screen strategy (see text for description). b, c, Bioinformatics classification of hits into biological process (b) and molecular function (c) categories. *Categories found enriched (P < 0.05) relative to all the genes examined in the RNAi screen. Only categories with ten or more members are displayed.

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The RNAi screen identified 283 HSFs and 22 HRFs (of which 273 and 21 respectively are novel; Supplementary Tables 1 and 2). The number of HRFs constituted 7% of the total host factors identified. The identification of (1) some of the known HSFs (vATPase, endosomal transport regulators) and HRFs (IRF3; ref. 11) of WNV infection, and (2) multiple components of macromolecular assemblies—for example, vATPase, the endoplasmic-reticulum-associated degradation (ERAD) pathway, focal adhesion complex (FAC)—validated the reliability of our approach and the *in vitro* model. A cellular map summarizing several screen hits classified into cellular compartments and broad functional association categories is provided in Supplementary Fig. 2.

Of the 283 HSFs, 195 (69%) and 193 (68%) could be classified using biological process and molecular function categories, respectively (Fig. 1b, c; Supplementary Tables 3 and 4). There was a significant enrichment of genes regulating intracellular protein trafficking, cell adhesion and processes associated with the transport of ions and biomolecules. The enriched molecular function

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SUPPLEMENTARY INFORMATION

Legends for Supplementary Tables 1 and 2.

Supplementary Table 1. RNAi screen identifies 283 human genes required for West Nile virus (WNV) infection. All of the identified 283 genes that qualified the selection criteria are arranged in alphabetical order. Column E shows how many individual siRNAs scored out of the four comprising the pool against each gene, when tested separately. Columns G, L, Q, V and AI: shows the fold reduction in WNV infection when the indicated genes were silenced with either individual siRNAs (G, L, Q, V) or pooled siRNAs (AI), and columns H, M, R, W and AJ are the corresponding Z-scores (standard deviation (SD) from the mean infection of control samples). The relative cell numbers (RCN, number of cells in gene silenced wells/number of cells in control) for G, L, Q, V and AI are shown in L, N, S, X and AK, and the corresponding SD are shown in J, O, T, Y and AL. Column AM shows fold reduction of dengue virus (DENV) infection (column AO shows the corresponding SD) when genes were silenced with pooled siRNAs. The RCN corresponding to AN is shown in AP (AQ shows the corresponding SD). 'NT' indicates not tested in the individual siRNA screen against WNV. 'NTD' indicates not tested against dengue virus.

Supplementary Table 2. RNAi screen identifies 22 human genes whose silencing enhances West Nile virus (WNV) infection. All of the identified 22 genes that qualified the selection criteria are arranged in alphabetical order. Column E shows how many individual siRNAs scored out of the four comprising the pool against each gene, when

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