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Previews

SARS-CoV-2 RNA: Exclusive friends and common foes

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Infection with SARS-CoV-2 sets off a molecular arms race between virus replication and host cell defense. In this issue of Cell, Flynn, Belk, et al. integrate an advanced large-scale RNA-centered approach with custom CRISPR screens to functionally characterize the interactome of the SARS-CoV-2 RNA genome during infection.

Viruses employ a wide range of strategies to replicate within cells. SARS-CoV-2 is a single-stranded, positive-sense RNA virus that coopts the host protein synthesis machinery for translation of its RNA genome. A range of cellular cofactors are engaged for efficient replication and production of progeny virions. At the same time, many host proteins function to detect and restrict virus infection. Extensive efforts have been undertaken to characterize SARS-CoV-2 proteins and their interactions with host factors, yet a gap remained in the understanding of how cellular factors interact with the viral genome. Recent studies have endeavored to elucidate the interactions between SARS-CoV-2 viral RNA (vRNA) and host cell proteins (Flynn et al., 2021; Labeau et al., 2021; Lee et al., 2020; Schmidt et al., 2021). In this issue of Cell, Flynn, Belk, et al. explore the vRNA interactome during SARS-CoV-2 infection using comprehensive identification of RNA-binding proteins by mass spectrometry (ChIRP-MS) and functionally analyze the identified RNA-host protein interactions (Figure 1) (Flynn et al., 2021).

The authors designed over 100 oligonucleotide probes, capturing the full length of the SARS-CoV-2 RNA genome, which they used to enrich for vRNA-interacting host proteins in two cell lines susceptible to SARS-CoV-2 infection. The use of two cell lines and extensive probe coverage enabled identification of 309 high-confidence interactors, expanding on the 104 RNA-binding host proteins identified in a recent RNA antisense purification and quantitative mass spectrometry (RAP-MS) analysis (Schmidt et al., 2021). Although both studies identified host factors in diverse functional categories that associated with the vRNA, there is little overlap between the vRNA-protein interactome and the viral-host protein-protein interactome (Gordon et al., 2020). This demonstrates that SARS-CoV-2 RNA and proteins interact with different sets of host proteins, an important distinction in understanding SARS-CoV-2 biology.

Given that neither of these RNA-centered investigations were performed in cells derived from the lung, Flynn et al. (2021) sought to validate the physiological relevance of their findings by analyzing previously published single-cell RNA sequencing profiles from primary human lung cells (Travaglini et al., 2020). In those data, they detected expression of >95% of their human ChIRP-MS hits in SARS-CoV-2 target cells, including lung epithelial club and ciliated cells and alveolar cells. Thus, the majority of the identified host factors may be relevant during SARS-CoV-2 infection of lung cells, warranting future investigations in human lung cell lines or more physiologically relevant models. Furthermore, defects in innate immunity in the two cell lines used, Vero E6 (due to lack of type I interferon production) and Huh7.5 (due to mutational inactivation of RIG-I), argue for confirmation studies in innate immune-competent models for a comprehensive understanding of the interplay between cellular antiviral immune responses and the SARS-CoV-2 genome.

To better understand whether the identified RNA-protein interactions were conserved or virus specific, Flynn, Belk, et al. placed their SARS-CoV-2 ChIRP-MS dataset into context with previously published ChIRP-MS data (Ooi et al., 2019) on three other positive-sense RNA viruses (dengue virus, Zika virus, and rhinovirus). They found that ribonucleoproteins robustly interacted with all four viruses, whereas SARS-CoV-2 was enriched for mitochondrial and proteasomal proteins. Many factors related to intracellular vesicles and trafficking strongly associated with SARS-CoV-2 vRNA, and some were conserved across all RNA viruses that were evaluated. This is consistent with intracellular membrane remodeling by positive-sense RNA viruses to generate replication organelles for protected vRNA replication and transcription.

The authors next sought to functionally characterize the vRNA-interacting host proteins by analyzing previous genome-wide CRISPR perturbation data (Wei et al., 2021). They bolstered those findings with a custom screen, targeting nearly all of the proteins in the SARS-CoV-2 RNA interactome, to define factors that functionally impact virus replication. This approach revealed that most of the identified RNA-binding proteins exert antiviral effects, suggesting that a notable proportion of the interactions between vRNA and host proteins function to restrict viral infection, as opposed to virally derived RNA hijacking host cell proteins for productive viral replication. Nonetheless, a smaller set of eight validated pro-viral proteins was identified, with SMARCA4, a transcription activator, as the top hit. Corroboration of these data with pharmacological inhibition of identified pro-viral proteins would validate their roles in viral infection and provide potential new directions for antiviral therapies. Along these lines, Schmidt et al. (2021) demonstrated that pharmacological inhibition of several vRNA-binding proteins identified in their RAP-MS study inhibited SARS-CoV-2 replication in two
susceptible cell lines, including a lung epithelial cell line.

The aggregated ChIRP-MS data showed that most of the validated antiviral proteins interact with other RNA viruses, suggesting a shared initial host response to vRNA that extends beyond well-known pattern recognition receptors. The authors conducted functional targeted CRISPR screens with six additional RNA viruses, including enveloped and non-enveloped viruses with positive- and negative-sense genomes, to compare functional conservation of SARS-CoV-2 pro- and antiviral factors. Interestingly, many proteins have shared antiviral roles against multiple viruses, and some are conserved across all seven viruses.
including coronaviruses and influenza A virus. In contrast, vRNA-interacting proteins with pro-viral roles were more often virus-specific, with many involved in viral entry.

Finally, the RNA-centered and CRISPR-based screening approaches established a functional connection between SARS-CoV-2 RNA and the mitochondria, particularly mitochondrial matrix proteins. Electron microscopy of Huh7.5 cells during SARS-CoV-2 infection revealed morphological changes to the mitochondria. The functional role of identified mitochondrial proteins was investigated by a targeted CRISPR approach in Vero E6 cells across seven RNA virus infections, and a number of mitochondrial proteins were found to antagonize replication of multiple viruses, consistent with a role for mitochondria as a platform for host innate immune responses against RNA viruses. Future studies in innate immune competent cells are warranted to decipher the interplay between interferon-mediated innate antiviral responses and the observed viral antagonism by mitochondrial factors.

Overall, Flynn, Belk, et al. provide a highly comprehensive landscape of host protein interactions with SARS-CoV-2 RNA during infection. Combined with the recent work of Schmidt et al. (2021), these studies highlight the wealth of information provided by RNA-centric investigations, complementing the growing literature on molecular and cellular mechanisms of SARS-CoV-2 pathogenesis. The integration of the SARS-CoV-2 ChIRP-MS data and targeted CRISPR studies in the context of other large datasets broadens our understanding of the initial cellular response to SARS-CoV-2 and diverse RNA viruses. The functional categorization of pro- and antiviral factors paves the way for detailed mechanistic studies to inform our understanding of virus-host interactions and enable the development of new antiviral strategies.

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The imitation game: How glioblastoma outmaneuvers immune attack

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Immune evasion and resistance to immunotherapy mark major roadblocks in treating glioblastoma, the deadliest form of brain cancer. In this issue of Cell, Gangoso et al. demonstrate that the immune microenvironment drives glioblastoma cells to hijack myeloid-characteristic transcriptional and epigenetic circuits as a mode of immune evasion.

Immunosurveillance and immune evasion are hallmark processes of cancer evolution and describe the complex interplay between tumor cells and their host immune microenvironment, determining tumor elimination or progression. Harnessing these interactions through immunotherapy has revolutionized clinical treatment for a variety of tumors. Amid these successes, glioblastoma (GBM), the most common adult brain tumor and one of the deadliest forms of cancer, has emerged as a prototype of immune evasion and resistance to immunotherapy (Jackson et al., 2019). Recent studies...