Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Cell
Leading Edge

CellPress

Preview

A Crisp(r) New Perspective on SARS-CoV-2 Biology

Adam L. Bailey[1,4,*] and Michael S. Diamond[1,2,3,*]
[1]Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO, USA
[2]Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA
[3]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA
[4]Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI, USA
*Correspondence: albailey@wisc.edu (A.L.B.), diamond@wusm.wustl.edu (M.S.D.)
https://doi.org/10.1016/j.cell.2020.12.003

Complementary genome-wide CRISPR-Cas9 screens performed by multiple groups reveal new insights into SARS-CoV-2 biology including aspects of viral entry, translation, replication, egress, and the genes regulating these processes. Comparisons with other coronaviruses enhances our understanding of the cellular life cycle of this medically important family of emerging viruses.

Over the past 5 years, CRISPR-based screening has fueled a wave of remarkable discoveries in cell biology, and virus-host interactions have been no exception (Puschnik et al., 2017). Many groups have generated cell lines transduced with libraries of small guide (sg) RNAs that cause insertions or deletions in a single gene per cell, disabling expression or function of the respective protein. Survival screens with cytolytic viruses conducted over a few weeks' period results in the outgrowth of cells resistant to infection and death. Although this approach cannot identify proviral factors that also are essential for cell viability, it is nonetheless a proven and robust approach for identifying many host factors required for viral infection (e.g., receptors), which is accomplished by sequencing of the gene-specific sgRNA in the surviving cells. In this issue of *Cell*, three new articles (Hoffmann et al., 2020c; Schneider, et al., 2020; Wang et al., 2020) add to a growing list of host proteins required for SARS-CoV-2 infection that have been identified using CRISPR-based survival screens (Daniloski et al., 2020; Wei et al., 2020). Collectively, these five groups used several different sgRNA libraries (Brunello, GeCKO, and GeCKOv2) and multiple human or monkey lines derived from lung, liver, kidney, and myeloid cells (aided in some instances by exogenous expression of human ACE2, the SARS-CoV-2 receptor) to generate a wealth of information on SARS-CoV-2 biology that will serve as a foundation for the field to study for years to come.

Several of the screens cross-validate one another's findings by identifying the same gene or sets of genes that encode for proteins forming discrete functional complexes or pathways within the cell. Although the precise role that many of these proteins have in the viral life cycle is undetermined, their presumptive subcellular location provides insight into the biology of SARS-CoV-2 (Figure 1). Beginning with SARS-CoV-2 attachment to the cell surface, several screens identified genes regulating steps in the biosynthesis of glycosaminoglycans (GAGs) in addition to the proteinaceous receptor, ACE2. GAGs are negatively charged polysaccharides that decorate the cell surface and likely enhance infectivity by attracting and concentrating viruses, which often contain exposed patches of positive charge, onto the cell surface. Notably, many of the additional SARS-CoV-2 attachment factors recently reported (e.g., neuropilin-1 and HDL-scavenger receptor B type 1) were not identified in these screens. Endosomal cathepsins (e.g., cathepsin L), which function to cleave and activate the SARS-CoV-2 spike protein and facilitate fusion of the viral and endosome membranes, were found in multiple screens along with the endosomal GTPase Rab7a. Notably, these genes were not identified as proviral factors in screens that utilized cells ectopically expressing TMPRSS2 (Wang et al., 2020), a serine protease on the plasma membrane that primes spike activation and promotes SARS-CoV-2 entry into cells via direct fusion, bypassing the endocytic entry pathway (Simmons et al., 2013). A number of proteins localizing to the endoplasmic reticulum (ER), the ER-Golgi intermediate compartment (ERGIC), and the Golgi apparatus also were identified by the screens as critical for virus-induced infection and cell death, reflecting the roles these compartments have in the translation of SARS-CoV-2 structural proteins and virion assembly. Among the ER host factors identified as important for SARS-CoV-2 infection is the relatively obscure protein TMEM41B, which Hoffmann and colleagues also defined as an essential host factor for viruses of the *Flaviviridae* family (Hoffmann et al., 2020c). TMEM41B shapes the ER membrane into structures conducive to the formation of viral replication factories. ER-localized proteins involved in cholesterol homeostasis (e.g., SREBP, SCAP, and MBTPS1/2) also feature prominently in several screens, although different groups identified both positive and negative regulators of cholesterol biosynthesis as antiviral. While this discrepancy could be explained by idiosyncrasies of the particular screens or cell types used, it highlights how poorly understood this host process is for the SARS-CoV-2 life cycle. Finally, several additional host factors were identified as important for the egress stage of the viral life cycle. While also relatively poorly understood, recent work identified lysosomal exocytosis as a pathway by which coronaviruses exit infected cells (Ghosh et al., 2020). In light of this, many of the seemingly disconnected "hits" identified across these screens—the exocyst complex, phosphatidylinositol kinases (PIK3C3, PICfyve), and other

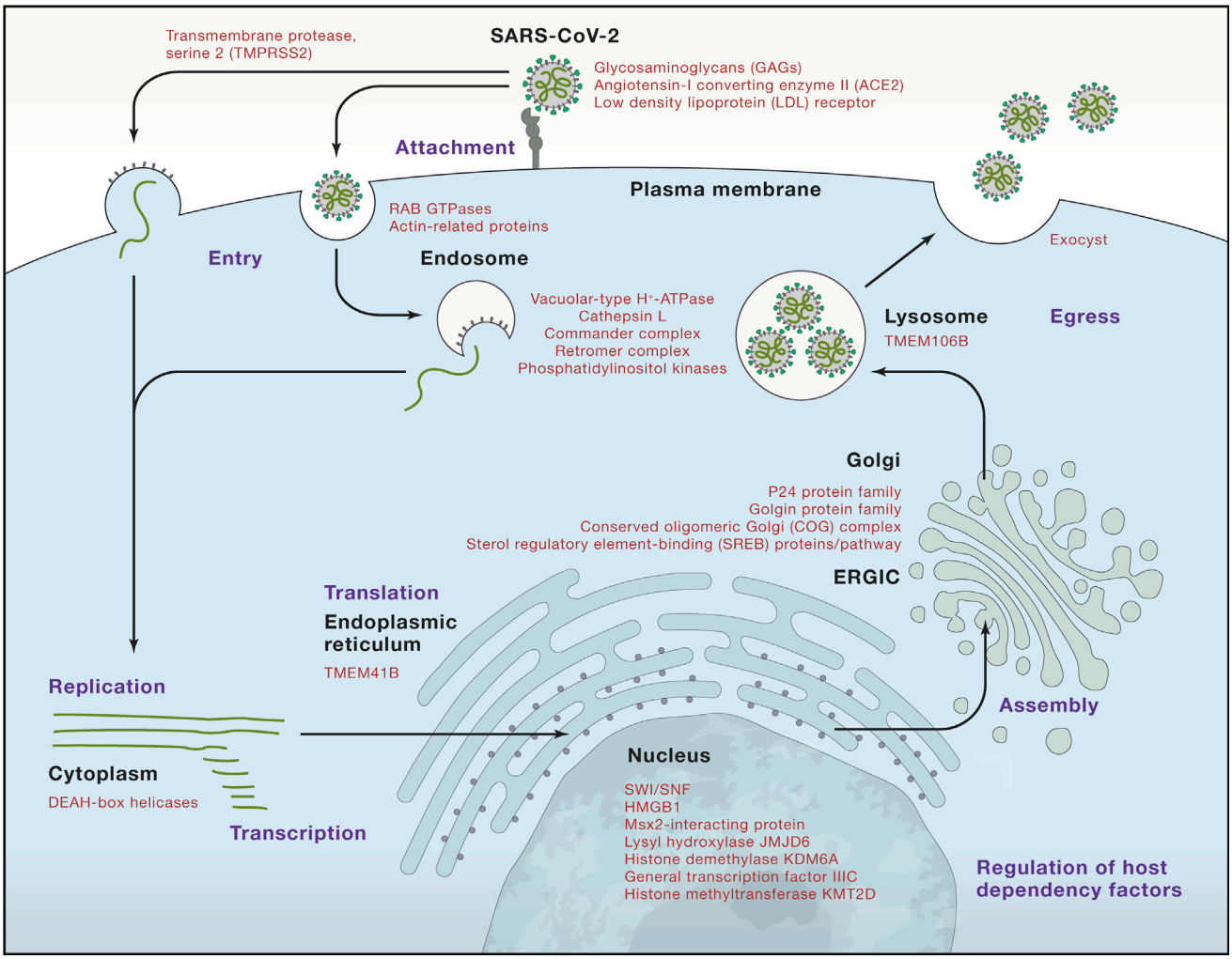

Figure 1. Proviral Host Factors Identified in Survival-Based CRISPR-Cas9 Screens

Proviral proteins (or multi-factor complexes) (red) are shown adjacent to their putative sub-cellular localization (black) and presumptive role in the viral life cycle (purple). Following engagement of the cell surface receptor, SARS-CoV-2 enters cells via two possible mechanisms: endocytosis or fusion of the virion membrane with the plasma membrane. In the cytoplasm, the RNA genome undergoes translation and replication, and sub-genomic RNA transcripts are generated. Structural gene translation occurs in the rough endoplasmic reticulum (ER), with virion assembly, maturation, and post-transcriptional modification occurring across the ER, ER-Golgi intermediate compartment (ERGIC), and Golgi network through a poorly understood process. Virions traffic to the cell surface via lysosomes and/or other vesicles capable of exocytosis. Endosomal recycling and interactions between the endosome and lysosome are thought to play roles in viral egress but are currently poorly defined.

pathways related to endosomal recycling (Commander complex, Retromer complex, TMEM106B)—may in fact function in virion internalization and/or egress via lysosomal exocytosis and/or other exocytic pathways.

The genes identified in these papers demand further study to achieve a comprehensive understanding of the SARS-CoV-2 life cycle. Such investigation may include distinguishing between factors that interact directly with viral proteins (e.g., SCAP, as suggested by Wang et al. [2020]) from those regulating viral protein interactions with other host proteins (e.g., SWI/SNF, as shown by Wei et al. [2020]). CRISPR-mediated survival screens in model cell lines are a beginning, as it will be critical to establish the relevance of these proteins and pathways to the biology of SARS-CoV-2 in primary human cells and ultimately in humans. For example, emerging data suggest that SARS-CoV-2 circulating in humans has a preference for TMPRSS2-mediated spike activation (Hoffmann et al., 2020a) and fusion at the cell membrane as the primary mode of entry in human lung epithelium. However, passage of SARS-CoV-2 in cell lines with low levels of TMPRSS2 (e.g., Vero cells) rapidly selects for variants with mutations in the furin cleavage site that enter cells via endocytosis. These two distinct entry pathways are not mutually exclusive and could vary by cell type *in vitro* and *in vivo* (Hoffmann et al., 2020b; Simmons et al., 2013). Nevertheless, the identification of endosomal host factors as proviral in screens that utilized cell lines with low levels of TMPRSS2 expression may require cautious interpretation until validated in primary cells.

Many of the gene products and complexes identified in the screens represent targets for host-directed anti-COVID-19 therapy. Indeed, several of the studies present data showing antiviral activity of compounds that target "hits" in the screens (Daniloski et al., 2020; Wang et al., 2020). While the genetic screens should prompt further interrogation of existing compounds and drugs that bind these proviral host factors, biochemical screens for small molecules targeting these pathways more specifically might yield novel classes of host-directed antivirals against SARS-CoV-2 infection. Finally, many of the studies reported comparative effects of specific genes on multiple different coronaviruses (and sometimes other viruses) in addition to SARS-CoV-2. Such an approach may identify shared aspects of virus biology that enables the development of broadly acting antivirals, which could help prepare us for the next viral pandemic, when it comes.

DECLARATION OF INTERESTS

M.S.D. is a consultant for Inbios, Vir Biotechnology, NGM Biopharmaceuticals, and the Carnival Corporation and on the Scientific Advisory Boards of Moderna and Immunome. The Diamond laboratory has received unrelated funding support from Moderna, Vir Biotechnology, and Emergent BioSolutions.

REFERENCES

Daniloski, Z., Jordan, T.X., Wessels, H.-H., Hoagland, D.A., Kasela, S., Legut, M., Maniatis, S., Mimitou, E.P., Lu, L., Geller, E., et al. (2020). Identification of Required Host Factors for SARS-CoV-2 Infection in Human Cells. Cell *184*, this issue, 92–105.

Ghosh, S., Dellibovi-Ragheb, T.A., Kerviel, A., Pak, E., Qiu, Q., Fisher, M., Takvorian, P.M., Bleck, C., Hsu, V.W., Fehr, A.R., et al. (2020). β-Coronaviruses Use Lysosomes for Egress Instead of the Biosynthetic Secretory Pathway. Cell *183*, 1520–1535.e14.

Hoffmann, M., Kleine-Weber, H., and Pöhlmann, S. (2020a). A Multibasic Cleavage Site in the Spike Protein of SARS-CoV-2 Is Essential for Infection of Human Lung Cells. Mol. Cell *78*, 779–784.e5.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.-H., Nitsche, A., et al. (2020b). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell *181*, 271–280.e8.

Hoffmann, H.-H., Schneider, W.M., Rozen-Gagnon, K., Miles, L.A., Schuster, F., Razooky, B., Jacobson, E., Wu, X., Yi, S., Rudin, C.M., et al. (2020c). TMEM41B is a pan-flavivirus host factor. Cell *184*, this issue, 133–148.

Puschnik, A.S., Majzoub, K., Ooi, Y.S., and Carette, J.E. (2017). A CRISPR toolbox to study virus-host interactions. Nat. Rev. Microbiol. *15*, 351–364.

Schneider, W.M., Luna, J.M., Hoffmann, H.-H., Sánchez-Rivera, F.J., Leal, A.A., Ashbrook, A.W., Le Pen, J., Ricardo-Lax, I., Michailidis, E., Peace, A., et al. (2020). Genome-Scale Identification of SARS-CoV-2 and Pan-coronavirus Host Factor Networks. Cell *184*, this issue, 120–132.

Simmons, G., Zmora, P., Gierer, S., Heurich, A., and Pöhlmann, S. (2013). Proteolytic activation of the SARS-coronavirus spike protein: cutting enzymes at the cutting edge of antiviral research. Antiviral Res. *100*, 605–614.

Wang, R., Simoneau, C.R., Kulsuptrakul, J., Bouhaddou, M., Travisano, K.A., Hayashi, J.M., Carlson-Stevermer, J., Zengel, J.R., Richards, C.M., Fozouni, P., et al. (2020). Genetic Screens Identify Host Factors for SARS-CoV-2 and Common Cold Coronaviruses. Cell *184*, this issue, 106–119.

Wei, J., Alfajaro, M.M., DeWeirdt, P.C., Hanna, R.E., Lu-Culligan, W.J., Cai, W.L., Strine, M.S., Zhang, S.-M., Graziano, V.R., Schmitz, C.O., et al. (2020). Genome-wide CRISPR Screens Reveal Host Factors Critical for SARS-CoV-2 Infection. Cell *184*, this issue, 76–91.