Editorial

The Host-Pathogen Ecosystem Viewed Through the Prism of Proteomics*

Ileana M. Cristea‡§

The coexistence and coevolution of hosts with pathogens is intrinsic to our ecosystem. Pathogenic infections induce a diverse array of changes in the hosts that are tightly linked to the progression of infection and establishment of disease. At the cellular level, this is reflected in alterations in host cell composition, organization, and ability to communicate with other cells. Thus, changes in the host proteome, metabolome, lipidome, and secretome have started to be recognized as signatures of infectious or disease states. These alterations function to either induce host defenses that counteract the infection or promote pathogen replication for spread of infection. Consequently, the discovery of these signature changes is an essential step for both understanding the biology of infection and identifying novel targets for therapeutic interventions.

This special issue aims to highlight and celebrate the contribution of proteomics to fundamental discoveries in infectious disease research. For over a decade, proteomic technologies, particularly mass spectrometry (MS)-based methods, have provided increasingly versatile tools that afford both breadth and depth of analysis of host cells during the progression on an infection. This versatility is demonstrated by the wide range of applications of proteomic methods for studying protein interactions, abundances, posttranslational modifications, subcellular localizations, and secretion that are temporally and spatially regulated during infection. These studies have led to the discovery of mechanisms that underlie pathogen replication or host defense, as well as the characterization of pathogen composition and features that contribute to its virulence. This issue tries to capture some of this diversity of infectious disease studies that have benefited from the integration of proteomics methods.

We start with a perspective article on the current state of proteomic technologies, discussing their ability to provide insights into the molecular basis of pathogen infection, their integration within multiomic studies, and their promise in assisting diagnostics and therapies (1). This is followed by several manuscripts that highlight the use of MS for defining the properties and virulence of pathogens. Ueberheide and colleagues focus on a prominent human bacterial pathogen, *Staphylococcus aureus*, showing that quantitative secretome information can be used as a predictor for the virulence of bacterial strains (2). Investigating the interaction surface between the bacterial pathogen *Streptococcus pyogenes* and human blood plasma, Malmström and colleagues demonstrate that absolute quantification via targeted mass spectrometry provides a platform for building a stoichiometric surface density model that allows the interrogation of factors that regulate bacterial virulence (3). Aiming to define properties of *Cryptosporidium parvum* that contribute to its inability to regulate protein folding in the endoplasmic reticulum (ER), Costello and colleagues used MS to identify Asn-linked glycans on pathogen glycoproteins, determining their preference for AsnXThr motifs and confirming their absence in the ER and Golgi (4). Further emphasizing the different landscapes of protein modifications observed in the proteomes of pathogens, the study by Yeh and colleagues investigated the prenylated proteome in blood-stage *Plasmodium falciparum* (5). Using enrichment with an alkyne-labeled prenyl analog and MS, this study does not only identify prenylated proteins, but also goes on to demonstrate that the prenylation state can regulate protein localization and can be a target for antimalarial drugs.

The next section of manuscripts illustrates the value of proteomics for understanding the diverse range of changes occurring in host cell composition upon pathogen infections. The perspective article by Alison McBride discusses the use of proteomic methods for studying DNA viruses known to trigger the development of human cancers (*i.e.* oncogenic viruses), and for identifying alterations in host cellular pathways that can be associated with infection and disease progression (6). Continuing the emphasis on the ability of DNA viruses to modulate host cells, the study from Weitzman, Garcia, and colleagues provides a large-scale, tour-de-force analysis of alterations in the proteome, phosphoproteome, chromatin-bound proteome, and histone post-translational modifications at different stages of infection of human fibroblasts with herpes simplex virus 1 (HSV-1)[1] (7). The Traktman and Terhune groups present a study focused on the human DNA virus, vaccinia virus (8). Integrating metabolic labeling,

From the ‡Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544

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[1] The abbreviations used are: HSV, herpes simplex virus; IFIX, interferon-inducible protein; MIB, multiplexed kinase inhibitor beads; HCMV, human cytomegalovirus.

phosphopeptide enrichment, and MS, this study aims to discover host substrates of the viral F10 kinase, a protein required for proper virion morphogenesis. Illustrating the value of this approach, the identified phosphorylation on the cytoskeleton regulatory protein mDia is shown to modulate actin stress fiber formation in an F10-dependent manner (8). Proteomic methods have been similarly applicable to characterizing the molecular details of infections with RNA viruses, and this is illustrated by the next three manuscripts. Using a SWATH-MS approach, Collins and colleagues investigated host proteome changes that occur upon infection with HIV-1 in human primary CD4+ T cells in culture, as well as in infected patients who have or have not undergone antiretroviral treatment (9). In agreement with the known ability of HIV-1 to suppress host immune responses, this study observed prominent perturbations in the type 1 interferon signaling pathway in both cell culture and patient samples (9). Viruses and hosts can also modulate the outcome of an infection by altering the composition of vesicles secreted by the cells, e.g. exosomes. This is well illustrated by Yuan and colleagues in their study of exosome composition upon infection with the important human pathogen, hepatitis B virus (10). Interestingly, exosomes contained proteasome subunits that were further shown to have the ability to regulate host immune responses. Other important drivers of host cell proteome changes during pathogenic infections are the extensive pathogen-host protein-protein interactions, which are temporally and spatially regulated throughout the progression of an infection. The complexity, challenges, and therapeutic promise of studying virus-host protein-protein interactions are highlighted in the review by Pietschmann and colleagues in the context of infection with flaviviruses, which include the important human pathogens Dengue, Zika, and hepatitis C viruses (11). Defining and characterizing virus-host protein interactions that occur at different stages of an infection has offered, and is expected to continue to provide, critical insights into mechanisms of virus entry, replication, assembly, and release.

The biological insights that can be gained from understanding changes in protein levels, processing, interactions, and modifications have been well demonstrated in studies of host defense mechanisms against pathogens. This is highlighted in this special issue by several manuscripts, starting with the perspective article by Overall and colleagues that emphasizes the need to understand the regulation of proteases by both host and pathogen factors in the context of infection (12). Given the role of proteases in regulating host immune responses, proteomic methods that can reveal proteolytically processed proteins (also termed degradomics studies) provide the opportunity to uncover molecular regulatory hubs at the interface between host defense and pathogen immune evasion. Additionally, this minireview provides a discussion of the therapeutic promise of targeting proteases (12). The manuscript by Nita-Lazar and colleagues adds to the theme of deciphering mechanisms of immune response regulation (13). Using proteome, secretome, and transcriptome analyses, this group of scientists identifies signature changes associated with the stimulation of Toll-like receptors and initiation of innate immune response. Matikainen, Nyman, and colleagues present another view of host immune response against pathogens via the formation of inflammasomes (14). Upon activation of inflammasome formation in response to Gram-negative bacteria lipopolysaccharide, MS revealed enrichment of inflammation-related proteins in the soluble secretome fraction. However, the vesicle-containing secretome contained components of the translational machinery, leading the authors to propose a model in which secretion and inhibition of translation plays a role in host defense against bacteria. Also, working on immune response, Crow and Cristea provide insights into the antiviral functions of the interferon-inducible protein IFIX in response to infection of primary human fibroblasts with the DNA virus HSV-1 (15). Using protein interaction studies, MS, microscopy, and functional assays, IFIX is found to have a dual role in defense via both stimulation of innate immune response and inhibition of virus gene expression. Furthermore, this study discovered that, similar to other virus immune evasion strategies, HSV-1 has already acquired a mechanism to suppress IFIX, further highlighting the importance of this host protein in defense against infection. Goodfellow and colleagues also elegantly demonstrate the value of quantitative proteomics for establishing a mechanism through which the highly contagious human RNA virus, norovirus, evades host immunity (16). MS analyses have been employed to discover changes in the composition of the eukaryotic initiation factor complex, which correlate with the suppression of translation of host interferon stimulated genes mRNAs and the activity of the viral protease NS6. To provide a robust, high-throughput platform for studying the activity of key phosphatases in innate immune response, Janes and colleagues describe a method integrating on-plate cellular fractionation, isolation of several phosphatases (*e.g.* STAT1, ERK2, JNK1), and quantification of enzymatic activity by ELISA (17). They demonstrate the usefulness of this method for monitoring relative phosphatase activity in specific subcellular compartments in response to infection of cardiomyocytes with coxsackievirus B3. The applicability of proteomic methods to a wide range of pathogen infections is beautifully illustrated by the study from the Cilia group. This study focuses on the Potato leafroll virus, which is transmitted from aphids to different plants (18). Using MS to compare the proteomes in the midguts of aphids attached to different plants (*i.e.* physalis *vs.* turnip), the authors demonstrate that plants have the ability to indirectly inhibit virus titers and transmission by modulating the aphid proteome, such as the turnip-specific targeting of several cysteine proteases at cell membranes.

Lastly, several manuscripts in this issue emphasize the push for proteomic platforms that can be used in a high-throughput fashion to discover biomarkers and therapeutic

targets. Moorman and colleagues implement a chemical proteomics method using multiplexed kinase inhibitor beads and MS (MIB/MS) to monitor kinase levels in cells infected with human cytomegalovirus (HCMV) (19). Given the wide-spread and pathogenicity of HCMV, as well as the lack of a vaccine or effective antiviral treatments against it, the authors propose that small molecules developed as drugs for treatment of other diseases may also be efficacious against HCMV infection, and therefore, repurposed as antivirals. Indeed, existing kinase inhibitors (that are in clinical use) were used to target kinases identified from the MIB/MS kinome profiling, and found to be effective at inhibiting virus titers. Another example of an effective proteomic platform for high-throughput screening comes from the study by Achkar and colleagues (20). Using the High-Density Nucleic Acid Programmable Protein Array (HD-NAPPA) technology, an array for the proteome of *Mycobacterium tuberculosis* was generated and used in rapid screening for antibody responses in sera from different patients. Analysis of sera from tuberculosis patients with and without coinfection with HIV led to the identification of known and novel possible biomarkers for tuberculosis.

Altogether, the manuscripts gathered in this special issue elegantly demonstrate that proteomics is well positioned to continue to make significant contributions to the field of infectious disease research. Looking ahead, this is an exciting time for studies that integrate these two fields of science. To the already proven value of proteomics in making discoveries in infectious disease research (21) can be added the ongoing developments of sensitive, accurate, and robust proteomic approaches. Consequently, this integration provides the opportunity to use these numerous experimental workflows to address a wide range of questions relevant to infectious disease biology, diagnosis, and therapy. The continued implementation of proteomics in pathogen infection studies is clearly needed. Numerous reports have demonstrated the ability to effectively compare the proteome composition of uninfected and infected hosts. However, so far, few studies have attempted to characterize these alterations in space and time, and to understand the dynamics of proteome organization during an infection. Similarly, the virus-host protein interaction networks remain unknown for the majority of viral proteins from human pathogens, and even less is understood about bacterial-host protein-protein interactions. Another aspect that deserves consideration is the dichotomy between the numerous findings obtained from large proteomic data sets and the relatively small number of findings that are pursued with functional analyses. This bottle-neck derives predominantly from time-constrains associated with functional studies and technical difficulties (*e.g.* absence of necessary reagents, cells, animal models). Therefore, in addition to improving the quality of proteomic data sets, further effort can be placed in developing experimental and interpretation methods that can bridge the intermediate space between large scale omic data sets and targeted functional analyses. Overall, we hope that this special issue will inspire future studies, and stimulate new questions and scientific endeavors at the interface between proteomics and infectious disease research.

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§ To whom correspondence should be addressed: Department of Molecular Biology, 210 Lewis Thomas Laboratory, Princeton University, Princeton, NJ 08544. Tel.: 6092589417; E-mail: icristea@princeton.edu.

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