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Comparative mapping of host–pathogen protein–protein interactions

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Pathogens usurp a variety of host pathways via protein–protein interactions to ensure efficient pathogen replication. Despite the existence of an impressive toolkit of systematic and unbiased approaches, we still lack a comprehensive list of these PPIs and an understanding of their functional implications. Here, we highlight the importance of harnessing genetic diversity of hosts and pathogens for uncovering the biochemical basis of pathogen restriction, virulence, fitness, and pathogenesis. We further suggest that integrating physical interaction data with orthogonal types of data will allow researchers to draw meaningful conclusions both for basic and translational science.

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Introduction

During the course of infection, pathogens use their proteins to hijack and re-wire a myriad of host biochemical processes — events that are required for efficient pathogen propagation. Therefore, characterization of host–pathogen protein–protein interactions (PPIs) greatly aids in the understanding of the mechanisms underlying pathogen replication. To date, several approaches have been employed to identify host–pathogen PPIs for viruses, bacteria, and parasites, including yeast two-hybrid (Y2H) and affinity purification coupled with mass spectrometry (AP/MS) [1–8] (Box 1). However, moving from systematic descriptions to functional/clinical relevance requires establishment of a genotype–phenotype relationship through the integration of global and reductionist approaches [9]. Usually, this can be accomplished through targeted characterization of interactions using secondary and even tertiary screens after initial, unbiased proteomic interrogation. In contrast, a comparative approach where PPIs are probed against functionally distinct genetic variants of a pathogen or host protein can yield biochemical insight into the observed phenotype and help to functionally prioritize host proteins. This approach, referred to as '**comparative proteomics**', leverages functional host and/or pathogen diversity to infer the biochemical basis for genotype–phenotype relationships. Examining PPIs between physiologically relevant genetic variants across the host–pathogen interface will provide a basis for uncovering molecular determinants of disease outcomes.

In this review, we highlight the importance of harnessing genetic diversity of both the host and pathogen when designing global proteomics studies. Comparative proteomics can explore the diversity of a population at the level of single nucleotide polymorphisms (SNPs) or as broadly as millions of years of evolutionary history, from the perspective of both the host and/or the pathogen (Figure 1a). Genetic determinants of virulence and/or pathogenesis have been described for several pathogens and comparative PPI mapping has the potential to uncover the underlying biochemical basis of these *in vivo* outcomes. Furthermore, comparing within or between species can highlight conserved and unique cellular pathways that are hijacked by pathogens (Figure 1b). In conjunction with orthogonal approaches, leveraging host/pathogen diversity with a comparative proteomics framework can greatly advance basic science and clinical goals (Figure 2).

The pathogen diversity axis

Genetic diversity among pathogens can directly impact host–pathogen PPIs and functional outcomes (Figure 1b), which has been demonstrated in several studies. For example, Greninger and colleagues explored the conservation of host–virus PPIs across several picornavirus 3A proteins [10•]. Picornavirus 3A remodels Golgi membranes into virus replication compartments and the host protein PI4KIIIβ, a regulator of Golgi membranes, is

Box 1 Detection of protein–protein interactions

Protein–protein interactions (PPIs) can be detected using yeast-two-hybrid (Y2H) or affinity purification/mass spectrometry (AP/MS). Y2H yields insights into pairwise PPIs, and takes advantage of baits and preys that are both linked to yeast transcriptional activators. When a bait successfully interacts with a prey, the transcriptional activators are brought together to drive yeast colony growth or reporter gene expression. Y2H technology has the advantage of scale and speed, as many baits can be screened rapidly once an appropriate prey library of complementary DNA (cDNA) has been created. In AP/MS, bait proteins are affinity purified using a bait-specific antibody, or via over-expression of affinity-tagged proteins. The resulting purified protein complexes are analyzed by MS to determine interacting prey. While more labor intensive, AP/MS is better suited to studying PPIs in the context of stoichiometric protein complexes. Unlike Y2H, preys detected by AP/MS are not subject to cloning-related biases and are present in their endogenous context. While low levels of endogenous expression may favor PPI detection by Y2H, AP/MS is better suited to studying PPIs for membrane proteins that may not effectively translocate to the nucleus for Y2H screening. Recent advances in MS, such as selected reaction monitoring, allow for a highly quantitative assessment of differential PPIs across diverse sets of sample that vary in host, pathogen or time [57]. Finally, when combined with double affinity purification of a viral bait and host prey, AP/MS has the added flexibility to be used to deduce other members of multi-protein complexes [6,20•].

required for the replication of diverse picornaviruses [11]. The authors utilized a comparative AP/MS approach with a diverse panel of picornaviruses and found that in most cases, the interaction of 3A with PI4KIIIβ was mediated by the acyl-CoA binding protein ACBD3. A panel of 3A point mutants was used to establish a positive correlation between the ability of 3A to interact with PI4KIIIβ and the efficiency of virus replication. This study also highlighted that distinct but functionally related proteins may mediate a shared host factor dependence on virus replication. Although PI4KIIIβ kinase activity is required for enterovirus 71 replication [12], its 3A protein was shown to recruit PI4KIIIβ via an interaction with ACAD9, another acyl-CoA binding protein, and not ACBD3 [10•]. Similarly, a systematic comparative PPI study of the lentivirus protein Vif revealed differential biochemical requirements for conserved pathogen protein function. Vif is an accessory factor that is required to counteract the host cytidine deaminase APOBEC3 [13]. In the absence of Vif, APOBEC3 is packaged into daughter virions and causes the hypermutation of viral genomes upon infection through the deamination of cytidines into uridines [14–17]. Vif binds to and degrades APOBEC3 through the recruitment of a Cullin-RING ubiquitin ligase complex [18]. Importantly, Vif-mediated degradation of APOBEC3 is conserved across the lentivirus family [19]. Through comparative AP/MS of different Vif lineages, Kane and colleagues demonstrated that primate lentivirus Vifs require the non-canonical host transcription factor CBFβ for complex formation, whereas non-primate Vifs required either no host co-factor, or a different non-canonical host co-factor, the cellular peptidyl prolyl isomerase CYPA [20•]. Thus, the Vif protein co-evolved with its hosts to hijack unrelated co-factors in order to counteract a potent cellular innate antiviral response. The authors further suggest that the use of CYPA as a non-canonical co-factor for Vif-APOBEC3 complex formation could potentially serve two purposes by disrupting both APOBEC3 and CYPA function, in a manner similar to the dual-hijacking of CBFβ by HIV-1 Vif [21].

Additionally, pathogen diversity can be used to reveal common themes for host–pathogen PPIs, as was done in a broad study of host–virus PPIs using 70 viral proteins from 30 distinct human viruses. Pichlmair and colleagues found that DNA viruses were specifically enriched for PPIs that link cell cycle to chromosome biology and transcription, whereas PPIs with RNA viruses are enriched for processes that degrade or detect viral RNA, both processes that are known to be important for the respective class of viruses [22]. In another notable study, Rozenblatt-Rosen and colleagues studied 123 tumor-causing virus proteins using AP/MS and demonstrated how different viruses can manipulate the Notch signaling pathway to influence cell proliferation via distinct host–pathogen interactions [23••]. Future proteomic studies of this nature that are more quantitative as well as systematic across larger families of pathogens will provide additional key information about the host–pathogen interface.

Furthermore, comparative approaches utilizing the significant diversity within a given pathogen species in virulence and/or pathogenicity afford the opportunity to uncover PPIs that correlate, and ultimately determine clinical outcomes in the host. In a pair of papers led by White and colleagues, the oncogenic E6 and E7 proteins from several strains of human papilloma virus (HPV) with differing cancer risks were subjected to AP/MS in an effort to identify differential PPIs that could contribute to tumorigenesis [24,25]. These studies are notable for their comprehensive nature and experimental design; however, future work remains to elucidate the biochemical determinants of tumorigenesis for high-risk HPV strains. In summary, by carefully selecting pathogen variants, from highly related strains to evolutionary distinct species, comparative proteomics can identify disease-specific PPIs and general classes of hijacked cellular pathways.

The host diversity axis

Host diversity can impact mechanisms of pathogen restriction, virulence, and fitness, and the resolution to examine this diversity can span from SNPs to phyla (Figure 1b). Diversity between hosts at a single gene can result in profoundly different host–pathogen PPIs and phenotypes, the most well known instance being the disruption of a PPI between CCR5 and the human immunodeficiency virus-1 (HIV-1) envelope protein. A 32 nucleotide deletion in CCR5 renders homozygous

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