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Comparative mapping of host–pathogen protein–protein interactions

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Abstract

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.

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

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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.

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•].

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 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 entero-virus 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, APO-BEC3 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 carriers resistant to HIV-1 infection [26], and is the basis for an anti-HIV-1 gene therapy currently in clinical trials [27]. At the species level, host-specific PPIs can result in pathogen restriction in one species, and permissivity in the other [28•]. For example, Ashour and colleagues showed that dengue virus (DENV) NS5 binds to and degrades human STAT2 to restrict antiviral signaling. However, this restriction is based on a species-specific host–pathogen PPI, as NS5 does not bind mouse STAT2, which is thus able to restrict DENV replication in mouse cells [29]. Global proteomic studies with pathogen virulence effectors conducted in disparate species are well suited to uncover functionally relevant targets of pathogen antagonists, such as STAT2 and DENV NS5, or alternatively, viral targets of cellular restriction factors such as TRIM5α and HIV-1 Capsid [30]. In contrast, conservation of host–pathogen PPIs across species can lead to insights into virulence. For example, Memisevic and colleagues discovered three previously unknown virulence factors of the pathogenic bacterium *Burkholderia mallei* by screening secreted *B. mallei* proteins for interactions with human and mouse proteins using Y2H. The mouse model of *B. mallei* infection recapitulates much of the pathophysiology observed in human infections, and the authors hypothesized that host–pathogen PPIs that are conserved between the two hosts may mediate virulence [31•]. This comparative approach filtered the number of potential virulence factors from 49 to eight, three of which resulted in reduced virulence *in vivo* when mutated. Finally, vector-borne pathogens, such as DENV, are ideal candidates for cross-phylum comparisons. In one such Y2H analysis, interactions were determined between DENV and its two hosts (human and mosquito), and 14 PPIs were conserved across host species [32]. While the function of these PPIs remains to be elucidated, these conserved interactions could facilitate replication in two divergent hosts and could serve as ideal drug

targets. These studies highlight how host diversity can be harnessed to systematically identify mechanisms of restriction, virulence and potentially fitness.

Coupling orthogonal datasets to advance functional understanding

No single approach is sufficient to fully characterize the host–pathogen interface, and PPI analysis is one of several complementary approaches that, when integrated together, can provide a more comprehensive network view of hijacked cellular processes across hosts and pathogens. Orthogonal technologies include monitoring post-translational modifications [33–36], gene expression profiling [23••,37•], and genome-wide genetic screening [38–42]. Moreover, as more host–pathogen PPIs are uncovered, common themes and differences will be revealed when studies are compared to one another [1]. Non-pathogen related disease datasets could also be used to provide functional insight into host–pathogen PPIs. For example, integration of host genetic data from The Cancer Genome Atlas (http://cancergenome.nih.gov/), which globally describes mutations in different types of cancer, could be used to highlight the importance of specific PPIs in cancer development by identifying common oncogenic pathways that are also targeted by cancer-causing viruses, including HPV, hepatitis C virus and Kaposi's sarcoma-associated herpesvirus (KSHV) [43]. Finally, visualization of integrated orthogonal datasets can help identify common themes, and software such as Cytoscape (www.cytoscape.org), STRING (http://string.embl.de), and NeXO (www.nexontology.org) are examples of intuitive tools for such a task. The integration of these data into network-centric models will undoubtedly lead to a deeper understanding of how pathogens rewire host cellular processes [44].

Analysis of host and pathogen genetics on evolutionary timescales can also provide insights into the evolutionary interplay between host and pathogen. For example, Jäger and colleagues found a significantly higher level of sequence conservation for human proteins that interact with HIV-1 as compared to the complete human proteome [6]. This observation suggests that HIV-1 targets essential cellular pathways via physical interaction with highly conserved host factors. In a different approach, Davis and colleagues leveraged the 1000 Genomes Project sequence data to identify signatures of recent positive selection in host factors that physically interact with KSHV-specific viral proteins [1]. Positive selection implies that these mutations confer a fitness advantage to the organism, and such signatures of evolution have been used to identify the biochemical basis of host restriction factor activity [45], and their antagonism by pathogens [46]. Thus, enrichment for positive selection in pathogen-interacting host factors suggests that interactions at these nodes may represent instances of host–pathogen antagonism, and such evolutionary analysis could be used to guide functional studies. Similarly, sequence variation among well-established clinical cohorts could be used to identify susceptibility or protective mutations that impact host–pathogen PPIs. Moreover, with the advent of induced pluripotent stem cell (iPSC) technology, disease-related haplotypes could be tested directly for differential host–pathogen PPIs in patient-derived iPSCs. Pathogen sequence databases could also be used to inform how highly conserved (or variable) residues impact host–pathogen PPIs (Table 1). Moreover, new methods such as CirSeq [47] will allow researchers to map dynamic fitness landscapes of pathogens by tracking the genetic composition of RNA virus populations over time, which can be used to inform PPI studies. When coupled with genetic interaction

mapping of well-characterized point mutants [48], mapping high and low fitness pathogen mutations to specific host–pathogen PPIs will provide significant functional and structural insight into the pathogen fitness landscape.

Caveats and potential solutions

To best capture physiologically relevant PPIs with available technologies, several caveats must be considered. While the caveats discussed here are by no means exhaustive, they represent some of the major limitations associated with comparative host–pathogen PPI mapping. PPIs are dynamic by nature and the context by which PPIs are probed can greatly influence results. Traditional PPI experimental approaches (Box 1) are best suited for detecting stable PPIs; however, weak and/or transient PPIs can also be captured using specialized techniques such as cross-linking mass spectrometry [49,50] or BioID, a novel proximity-based biotinylation method [51•,52], in combination with AP/MS. Pathogen replication is itself a dynamic process and comparative host–pathogen PPI mapping across the timescale of pathogen replication or in specific cellular compartments could be used to infer temporal and/or spatial roles of host–pathogen PPIs. Cristea and colleagues recently demonstrated that time-dependent host–pathogen interactions could be captured during Sindbis virus replication by AP/MS [53,54]. Similarly, Moorman and colleagues identified spatiotemporally regulated PPIs during human cytomegalovirus infection involved in virion assembly [55]. As new quantitative mass spectrometry techniques such as selected reaction monitoring or data-independent acquisition are combined with AP/MS [56,57], spatial-dependent and time-dependent host–pathogen PPIs can be quantified during pathogen infection.

One of the greatest limitations for probing host–pathogen PPIs relates to the cellular context in which these PPIs are examined. Some interactions across the host–pathogen interface may only exist in the context of infection or in select cell types. While ideal, genetically inserting an affinity-tag into a pathogen genome and examining PPIs during natural infection is not possible in many cases. However, recent advances in genome engineering technologies such as CRISPR (clustered regularly interspaced short palindromic repeat) now make it possible to affinity-tag host proteins in their natural context [58], allowing for the examination of host–pathogen PPIs during infection. Both of these approaches simultaneously eliminate the possibility of artifacts introduced via over-expression of affinity-tagged proteins and could be used to inform analysis of over-expression PPI datasets. Finally, differential expression levels of bait proteins between cell or tissue types could confound comparative PPI results, but global protein abundance quantification could help correct these biases. Importantly, careful targeted experiments should be used to address whether a differential host–pathogen PPI is the result of a meaningful biophysical difference in the binding interface, or differential gene expression.

Conclusions

Our understanding of genetic diversity and our ability to quantitatively track changes in PPIs is rapidly improving with the development of new technologies, such that comparative mapping of host–pathogen PPIs has the potential to transform how genotype–phenotype

relationships are linked to the underlying biochemical interaction. By taking advantage of host and pathogen diversity, researchers can now conduct studies to identify conserved and differential interactions, which will ultimately offer insight into the basic biology of pathogenesis, and lead to more effective therapeutics.

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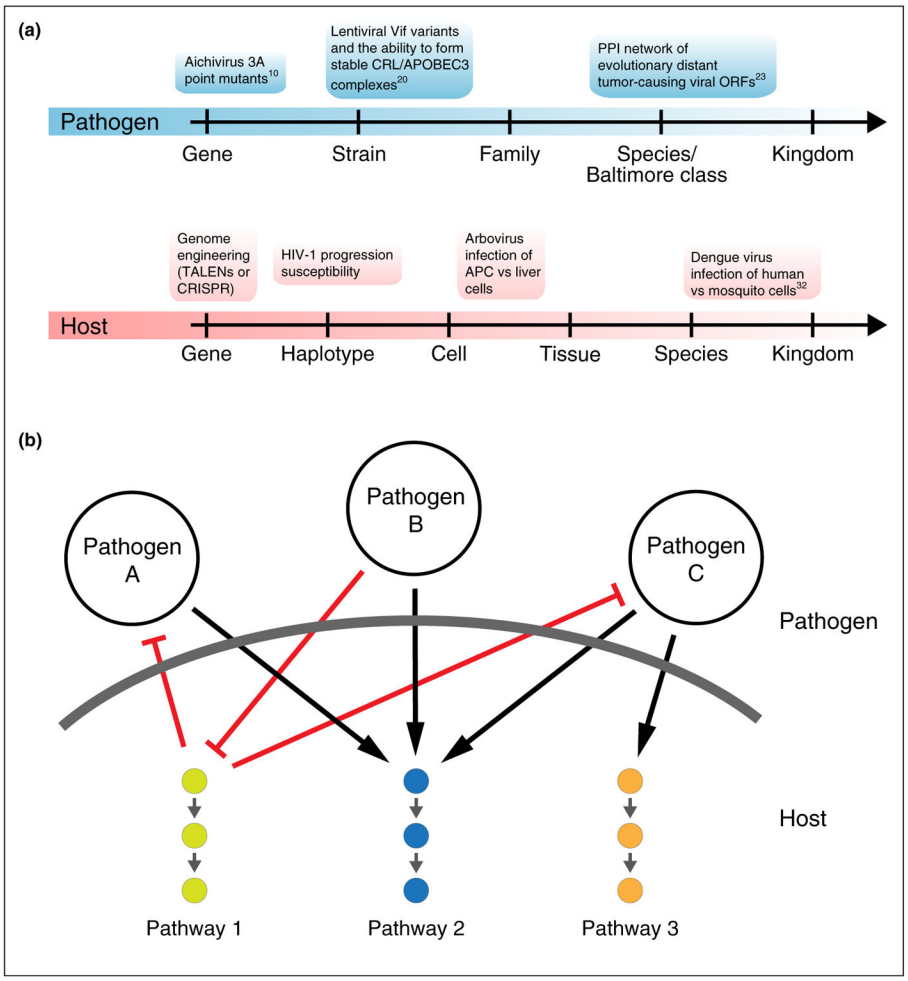

Figure 1.

Comparative PPIs: genetic diversity impacts host–pathogen PPIs. **(a)** Resolution of pathogen or host diversity variables for comparative PPI study design. Each class of experimental variable (pathogen or host) is ordered according to resolution or complexity. Along each scale are either published examples (referenced) or hypothetical comparative PPI experiments. The examples listed are by no means exhaustive. **(b)** Representation of host–pathogen comparative approach. The interrogation of how pathogen diversity can interact with the host can highlight unique and shared cellular pathways. This can manifest itself as inhibitory (red lines), or as promoting replication (black arrows). *Abbreviations*: CRL: cullin-RING ubiquitin ligase, TALEN: transcription activator-like effector nuclease, CRISPR: clustered regularly interspaced short palindromic repeat, APC: antigen-presenting cell.

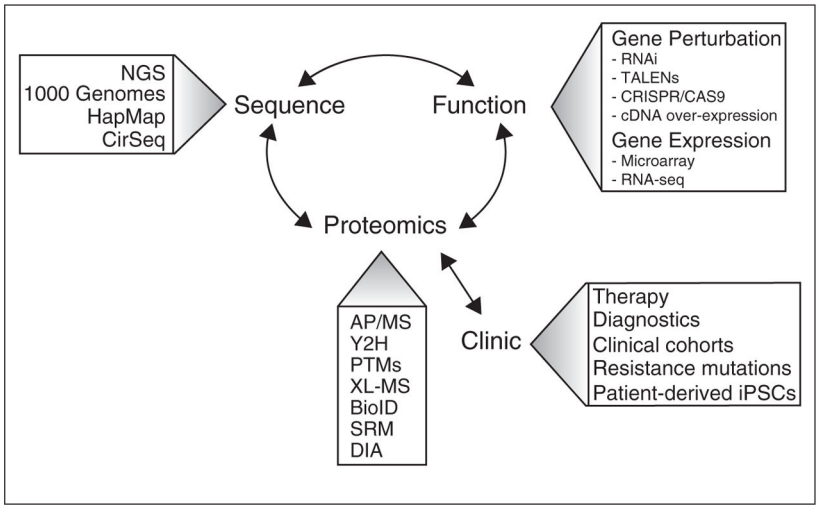

Figure 2.

Using comparative proteomics to move from systems to function. Building and refining systems models of biological processes requires the integration of orthogonal tools and datasets. The feedback between systems and reductionist approaches greatly improves the resolution of models of basic biological processes and strategies for translating these discoveries to the clinic. Using sequence diversity to inform comparative PPI studies can facilitate the translation of biochemical data into therapies. *Abbreviations*: PPI: protein–protein interaction, NGS: next generation sequencing, 1000 Genomes: http://www.1000genomes.org, HapMap: http://hapmap.ncbi.nlm.nih.gov, CirSeq: circular sequencing, RNAi: RNA interference, TALEN: transcription activator-like effector nuclease, CRISPR: clustered regularly interspaced short palindromic repeat, AP/MS: affinity purification mass spectrometry, Y2H: yeast-2-hybrid, XL-MS: cross-linking mas spectrometry, SRM: selected reaction monitoring, DIA: data-independent acquisition, PTM: post-translation modification, iPSCs: induced pluripotent stem cells.

Table 1

Complementary pathogen databases. Several publicly available databases compile a variety of systems-level analysis, including host–pathogen interactions, pathogen sequence diversity and protein structures.

Tool	Description	Website
EuPathDB	Database portal for non-viral eukaryotic pathogens with access to genomic-scale data deposited in other databases (e.g. PlasmoDB, ToxoDB, etc.)	http://eupathdb.org/eupathdb/
FLAVIdB	Database of antigenic data of flaviviruses	http://cvc.dfci.harvard.edu/flavi/
HIV systems biology [44]	Collection of web-based tools for exploring basics of HIV lifecycle, gene enrichment analysis, and viral association studies	http://hivsystemsbiology.org/
Influenza Research Database	Collection of influenza sequences, structures, immunological and host factor data	http://www.fludb.org/
Los Alamos National Lab HIV sequence database	Authoritative collection of HIV sequence variants	http://www.hiv.lanl.gov/
PlasmoDB	Comprehensive database of systems data with user friendly data mining tools for various Plasmodium species	http://www.plasmodb.org/
VirusMentha	Resource of virus-virus and virus-host PPIs	http://virusmentha.uniroma2.it/
NCBI Viral Genomes	Database of viral and viroid genome sequences	http://www.ncbi.nlm.nih.gov/genome/viruses/