

## REVIEW ARTICLE

# BEHIND THE SCENES OF HOST–MICROBE INTERACTIONS

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### Summary

Interaction between a host cell and pathogen is a permanent event and can have either adverse outcome leading to disease or great benefit for their mutual co-existence. Understanding pathological host–pathogen interaction is a prerequisite for unveiling the strategies of pathogens virulence. A number of methods exist today for deciphering and characterizing host–pathogen interaction. To increase their sensitivity and accuracy, these methods are commonly used in combinations, such as affinity purification and liquid chromatography–mass spectrometry analysis, cross-linking together with liquid chromatography–mass spectrometry analysis, or stable isotope labeling with amino acids in cell culture with affinity purification. In this review, we focus on study of the early interaction time interval when the pathogen binds and invades the host cell and activates sophisticated mechanisms to overcome the host defense barrier. We briefly describe the methods applied in identifying bacterial–host cell protein interactions while emphasizing these methods' various strengths and weaknesses.

*Key words: host; microbe; pathogen; protein–protein interaction.*

## INTRODUCTION

Host–microbe interaction is a term expressing the relationship between a host cell and a microorganism. The host cell usually is represented as a human cell, but microorganisms encompass bacteria, viruses, prions, and fungi. However, this relationship is not always harmful either for the host cell or for the pathogen itself. Firstly there is symbiosis represented by commensalism (e.g., gut microbiota), and mutualism. These relationships bring certain benefits for one or both organisms. On the other hand, there can be relationships that are harmful and undesirable for the host cell (parasitism) and could lead to infection (Fig. 1) (1,2). Host and pathogen influence one another always for worse. The interplay between the two organisms is not so simple as mere physical interaction. It is a whole process involving various mechanisms and diverse interactions based upon protein–protein interaction or protein–lipopolysaccharide interaction, where the lipopolysaccharide is a dominant component of the host's cellular surface. In this review, we focus on protein–protein interactions between host cell and pathogen, methods suitable for detecting those interactions, as well as the pros and cons of those various methods.

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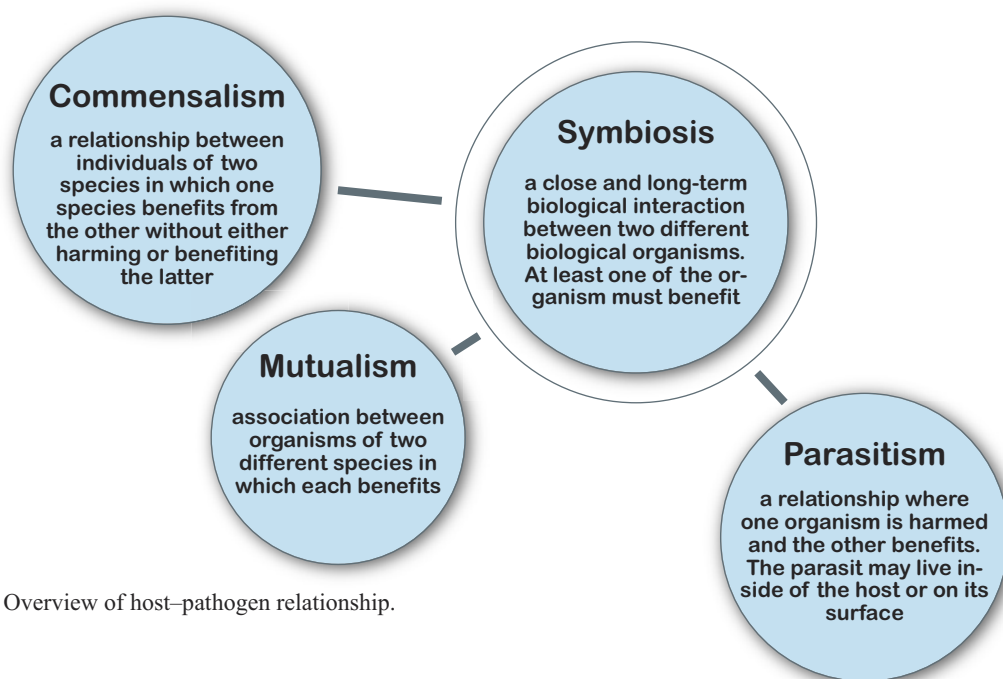
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## TARGET TOOLS OF INFECTION PROCESS - HOW TO GET THROUGH?

Before a pathogen can begin the infection process within the host organism it needs to overcome the particular defense mechanisms of that host. Once the pathogen is inside the host, it can inhabit one of two distinct niches during the infection process: extracellular space (including body fluids) or an intracellular niche inside the target cell.



**Figure 1.** Overview of host–pathogen relationship.

### First line of host defense

The first line of host defense against microorganisms is the mechanical barrier consisting of skin and mucous membranes. There exist also chemical barriers characterized by the likes of antibodies secretion, low pH in the stomach environment, and secretion of lysozyme and/or other cationic antimicrobial peptides, such as hemocidins, defensins and cathelicidins (3). A potential entry point for a whole range of microorganisms is created by an occurrence of non-intact, mucous membrane or wounded skin. Mucous membrane produces and secretes different substances (e.g., mucus) containing numerous glycoproteins known as mucins (4). Microorganisms coated in mucus may be prevented from adhering to the epithelium and ultimately eliminated by ciliary movement (5). Presence of the antibody immunoglobulin A on the mucosal surfaces is another crucial element in host immune protection (6). Overcoming this first line of host defense can be considered a key mechanism within the process of virulence for a wide range of pathogens.

Pili and fimbriae belong to group of bacterial type of adhesins. These proteinaceous filaments represent the first bacterial structures which mediate a physical contact between the pathogens and the host cells. This contact leads to cell signaling events and triggers the host immune responses. On the other hand, pathogens may be able to exploit this close interaction while secreting various effector proteins in order to manipulate the adverse milieu of the host cell (7–9). All adhesins display high selectivity for target molecules localized on the surface of the host cell (7). Every single process occurring after adhesion depends upon the type of cells that was attacked by the pathogen. Phagocytic cells, represented by macrophages, neutrophils, and dendritic cells, recognize pathogens through various pattern recognition receptors (PRRs). The result of such interaction is that the pathogen is ingested and a phagosome vesicle is formed. In cases of non-phagocytic cells (e.g., epithelial cells), a pathogen can employ a specific manner of entering the host. Such manners involve specific host cell functions associated with the cytoskeletal dynamics and vesicular trafficking. Typical for bacterial pathogens are “zipper” and “trigger” mechanisms for invading host cells by the activation of signaling cascades and reorganization of actin cytoskeleton (10–13). Viruses employ endocytosis by clathrin-dependent or clathrin-independent pathway as their mode of entry into host cell (10–13).

## Immediate recognition of microbes

The whole recognition process begins by recognition of pathogen-associated molecular patterns (PAMPs) of microbes through such PRRs as, for example, members of the toll-like receptors (TLRs) family in professional phagocytic cells (Tab. 1). Dimeric complexes of TLR2 with TLR1 or with TLR-6 recognize bacterial lipoproteins, glycolipids, lipoarabinomannans, some bacterial lipopolysaccharides, and proteins (e.g., stress proteins). The ligand for TLR3 is double-stranded RNA. TLR4 dimer binds lipopolysaccharide, stress proteins, or some viral proteins. TLR5 recognizes flagellin, which is a component of bacterial flagella and some types of bacterial secretion systems. TLR7 and TLR8 ligands are the nucleic acid-like structures of viruses, and TLR9 serves as receptor for fragments of prokaryotic DNA (e.g., non-methylated CpG motifs). The ligands for some TLRs have not yet been identified. There also are TLRs that appear to be restricted to just certain vertebrates. TLR11, for example, is expressed on murine cells but not on human cells (14,15). Numerous TLRs exist that are not expressed exclusively on cell surface membrane. TLR3, TLR7, TLR8 and TLR9, for example, recognize ligands that arise mostly from disrupted microbes in the endosomal compartment (16–19).

Opsonins, including complement proteins and antibodies, also are involved in the host–pathogen interaction. The complement system is the main humoral component of the innate immune response and consists of more than 50 serum-circulating proteins. The function of opsonins is to facilitate phagocytosis of microorganism via binding to specific receptors on the surfaces of phagocytic cells like complement receptor 3 (20,21) and Fc-gamma receptors (22,23).

The recognition of microbes by all these innate receptors triggers an intracellular signaling cascade that leads to the activation of antimicrobial effector mechanisms to promote killing and destruction of the invader (24,25). After a pathogen is engulfed by a professional phagocyte, the process of phagosome maturation starts immediately. During this maturation, the phagosome sequentially fuses with early endosomes, late endosomes, and, finally, with lysosomes wherein the pathogen can be killed (26,27). This process is characterized not only by remodeling of the membrane but also by changes inside the phagosomes, which acquire degradative and microbicidal features, such as highly oxidative products (e.g., reactive oxygen species or reactive nitrogen species) and highly acidic pH levels (28,29). When bacteria interfere with the classical destructive endosomal pathway, the professional phagocytes employ the alternative mechanism of their elimination known as autophagy (30,31). This effector mechanism functions as an intracellular innate defense pathway in response to infection by a variety of bacteria and viruses (32). Although autophagy is fundamentally a „self-cleaning process”, it also facilitates the degradation of pathogens as a xenophagy process (33,34). In this case, similarly as does a phagosome, the autophagic vacuole (which is an autophagosome surrounded by double membrane) fuses with the lysosomes, thereby giving rise to an autolysosome with capacity to kill and destroy the microbe (31,35). Even in the cytosol bacteria are not protected from immunological surveillance. They are recognized by the cytosolic receptors known as nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), AIM2-like receptors (ALRs), or retinoic-acid-inducible gene I RIG-I-like receptors (RLRs). After binding of either PAMPs (in the case of NLRs) or nucleic acids (in the cases of ALRs and RLRs), these cytoplasmic receptors contribute to the initiation of defense immune response (36–40).

**Table 1.** Overview of TLR receptors with their ligands and localization in cell.

TLRs	TLR ligand	Localization
<b>TLR1/TLR2</b>	glycolipids, lipopolysaccharide, bacterial lipoproteins, lipoarabinomannans, stress proteins	cell surface
<b>TLR2/TLR6</b>	glycolipids, lipopolysaccharide, bacterial lipoproteins, lipoarabinomannans, stress proteins	cell surface
<b>TLR3</b>	double-stranded RNA	endosome
<b>TLR4</b>	lipopolysaccharide, stress proteins, some viral proteins	cell surface
<b>TLR5</b>	flagellin	cell surface
<b>TLR7</b>	nucleic acid-like structures of viruses	endosome
<b>TLR8</b>	nucleic acid-like structures of viruses	endosome
<b>TLR9</b>	fragments of prokaryotic DNA	endosome

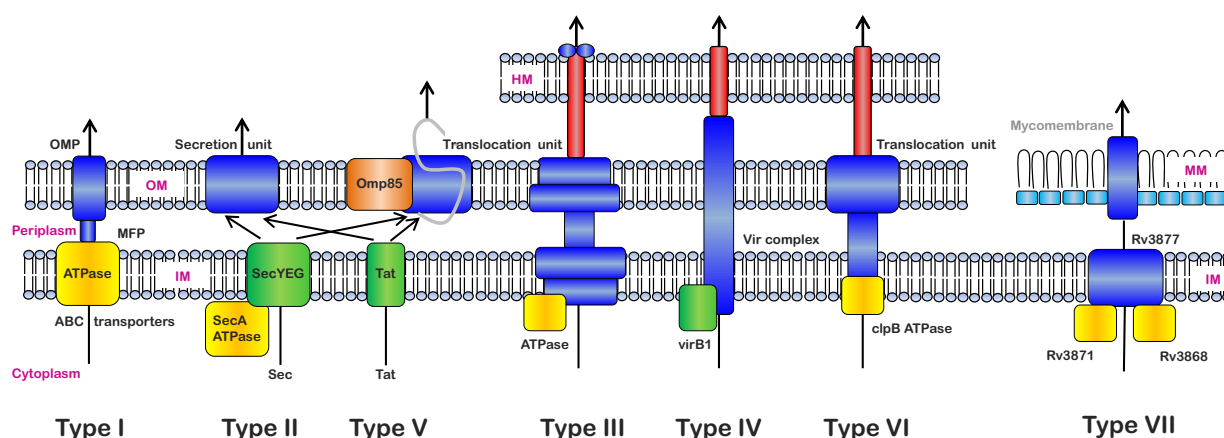
## Inside the host cell

In the cytosol of the host cells, bacteria seek out a friendly niche rich in nutrients for subsequent specific purposes in order to survive, replicate, and spread further. This shelter also protects them against intracellular microbicidal agents. Pathogens essentially can persist within the target cells in two different cell regions: the cytosol (and thus are known as cytosolic bacteria) or in the vacuole (thus termed intravacuolar bacteria) (41). In most circumstances, professional phagocytes can kill the pathogens inside lysosome vacuoles. However the intracellular bacteria can successfully bypass the terminal stage of phagolysosome and escape to the cytosol. A whole range of different pathogens are able to escape from the vacuole and/or phagosome by enzymatic lysis of membrane (42–45). Others are capable even of living inside the lysosomal-like compartment due to abrogation of its acidification (46). Some pathogens can inhibit the process of phagosome maturation (47,48), providing that the apoptotic or necrotic process within the host cell is modified (49–51).

Pathogens like *Escherichia coli* (*E. coli*) (52), *Campylobacter jejuni* (53), *Mycobacterium bovis* BCG (54), some viruses such as SV40 virus (55,56) and HIV (57), as well as some parasites (58) or toxins (59,60) can hijack the lipid rafts or lipid metabolism in the host cell in order to help themselves to enter the phagocytes or even the host cell (61,62). In this event, the proteins of lipid rafts can be detected in the phagosome and they play an irreplaceable role in indicating the stage of a phagosome's maturation or in its acidification (63). There is but limited information about nutritional content of the host cytosol during ongoing infection. Nevertheless, different bacterial genes and growth requirements have been identified as crucial for bacterial cytosolic replication and growth (41).

## Secretion systems and quorum sensing

The prokaryotic organisms exhibit the unique ability to transport their own proteins outside the cell or directly into the cytoplasm of the host cell. This process is called protein secretion and the secreted proteins are mainly responsible for virulence, hence they are usually named virulence factors. Except virulence factors, different small molecules and DNA are secreted through secretion system. During the infection of target cell, the secreted proteins mediate a number of divergent functions associated with the growth of bacteria, responses to surrounding environment, and/or the bacterial adhesion process. They might also manipulate the host cell in other ways. The protein secretion occurs through specific complex structures called secretion systems. To date, at least eight specialized secretion systems have been identified. (Fig. 2) (64,65). These are named by the following convention: type I secretion system (T1SS), T2SS, T3SS, T4SS, T5SS, T6SS, T8SS and T7SS which is unique for pathogenic *Mycobacteria* spp. Recently, T9SS has been characterized in phylum *Bacteroidetes* (66). The structural distinctions among these secretion systems reflect the multiplicity of phospholipid membrane types (67).

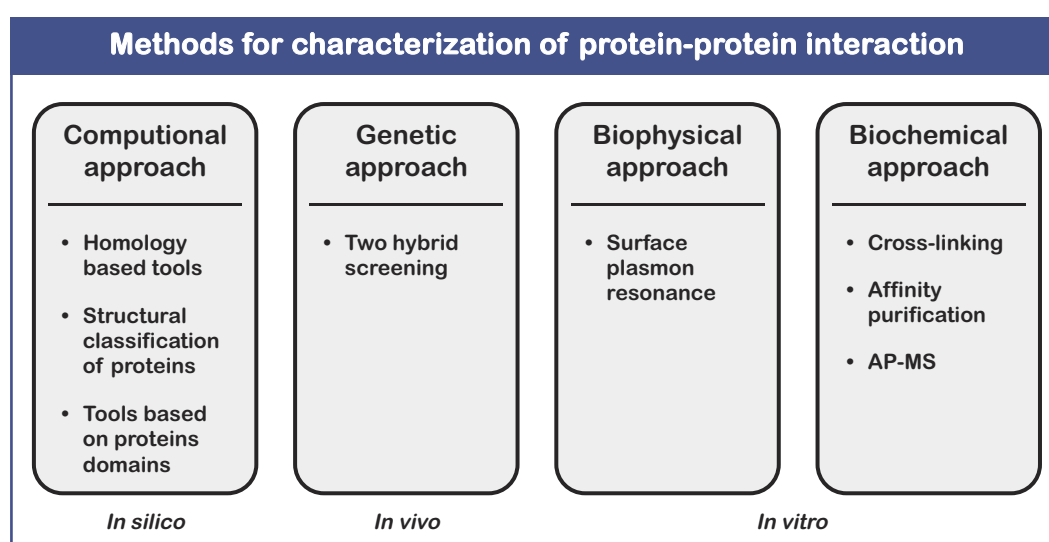


**Figure 2.** Secretion system of bacteria. T2SS and T5SS deliver proteins in two separate steps. Proteins are first secreted through Tat or Sec into periplasm and then are transferred across the outer membrane. T3SS, T4SS, and T6SS are able to transport virulence factor across three phospholipid membranes to the host cell. HM: host membrane; OM: outer membrane; IM: inner membrane; MM: mycomembrane; OMP: outer membrane protein; MFP: membrane fusion protein. ATPases and chaperones are shown in yellow. Taken from (71).

How do bacteria know when they should secrete the virulence factors? The explanation lies in the ability of both Gram-positive and Gram-negative bacteria to communicate through extracellular signaling molecules known as autoinducers. This process is also known as quorum sensing (68,69). Bacteria secrete these molecules into the environment, and, as the bacteria population grows, they gradually increase in concentration. Upon reaching a certain concentration threshold, the bacterial population can activate corresponding response genes that regulate various behaviors, such as virulence, biofilm formation, sporulation, bioluminescence, horizontal gene transfer, or antibiotic production (70).

## PROTEIN–PROTEIN INTERACTIONS

In order better to understand the molecular mechanism of pathogenesis, we must have deeper insight on the protein level and uncover the network of bacterial–host protein–protein interactions. The sum of all protein–protein interactions (PPIs) within a living cell is called the interactome (72). To study these interactions is a challenging process because it is a dynamic process and therefore some of those interactions occurring are transient or are located only in certain cellular environments. Currently, there exist a number of computational prediction methods or experimental methods (Fig. 3) for deciphering these intricate protein–protein interactions. Every method has its own advantages and disadvantages with regard to its sensitivity and specificity. Therefore, these various methods must usually be used in combinations to characterize, validate, and confirm the interacting proteins. This means it is critically important to design experiments very precisely.



**Figure 3.** Overview of methods for characterization of protein–protein interaction.

### Computational approach in characterization of protein–protein interactions

Computational prediction of PPIs could compensate for blind spots of experimental methods or it can be used to compile a basic outline of PPIs for further verification by experimental methods. The computational approaches are very extensive, because scientists always work with tremendous amounts of divergent data obtained from distinct databases and usually they combine two or more approaches for prediction purposes. In order substantially to increase the probability that a predicted interaction actually occurs and to reduce false positive rates, it is recommended that predicted candidates be filtered based upon their biological functions, cellular localizations, and expression profiles (73,74). The methods for predicting host–pathogen PPIs include interologs (75,76) and homology-based tools (74), structural classification of proteins (77,78), and prediction based on protein domains (79,80). Computational analyses usually result in enormous numbers of possible interactions which are gathered in different databases (e.g., STRING (81), DIP (82), MiMI (83), iPfam (84), VirHostNet (85), and IntAct (86)). These databases can also be used as template databases for searching out new PPIs. Modern software makes it possible to visualize simple and small networks

in specialized graphs wherein target proteins are represented as nodes and interactions between any two proteins are depicted as lines between the nodes (87,88). Networks of larger size and complexity usually are visualized using clustering approaches. Proteins termed bottlenecks and hubs can be identified and defined in these networks. Bottlenecks are defined by their large degree of betweenness, which describes the frequency with which a node lies on the shortest path between other nodes, and these are the central proteins for many paths within the network. Hubs are proteins with many interacting partners. Moreover, Yu and McDermott assume that these proteins could play important roles as virulence factors (89,90). All the main knowledge about non-interacting proteins can be found in a database known as the Negatome (91). This database is the critical point for training the various PPI prediction algorithms and for introducing gold-standard datasets of positive interactions.

An investigation for potential PPIs of *E. coli*, *Salmonella enterica* serovar *typhimurium*, and *Yersinia pestis* with *Homo sapiens* was done by Krishnadev. His team employed the homology detection approach. First they queried the DIP (Database of Interacting Proteins) database, which lists published interactions from large-scale two-hybrid screens, and homologous proteins were then aligned. Secondly, sequences of proteins encoded in both organisms were assigned in the iPfam database of protein family and domain interactions to reveal protein pairs based on domain–domain interaction. To narrow down the number of potential PPIs, the unlikely interactions were filtered out by localization information according to the pathophysiological niche of the pathogen and prediction of transmembrane regions (74). Huo et al. identified hundreds of predicted proteins pairs in a study of host–pathogen interaction between *Mycobacterium tuberculosis* and *Homo sapiens*. Systematic workflow for that prediction was based on sequence motifs. Protein sequence to predict homologs by the interolog method were selected as initial inputs. The identified interacting partners were then filtered based on domain–domain interaction to predict PPIs. Knowledge about any given protein’s subcellular location, tissue specificity, biological process, molecular function, and cellular component was then used in a final filtering step. All listed interactions have been kept in the PATH (Protein interactions of *Mycobacterium tuberculosis* and Human) database (79). Schleker et al. predicted the *Salmonella*–human interactome using sequence identity or domain alignment. The predicted interactions were further compared with known *Salmonella*–host PPIs. Those authors also focused on predicted binding partners of *Salmonella* effector proteins and outlined their roles in host defense responses (75).

### Genetic approach in characterization of protein–protein interactions

The two hybrid screening was the first method to enable detection of PPIs in a living organism and it could be automated for high-throughput studies (92). Compared to *in vitro* experiments or bacterial expression systems, the two hybrid screening is much more suitable because yeast is more similar to higher eukaryotes (93). When experiments are designed, there is a choice of two different approaches – library or matrix – that can be applied. In the library approach, prey is represented by cDNA or open reading frames (ORFs). In the matrix approach, a collection of defined preys is used instead of a random collection of ORFs (94,95). The screening is then based on the interaction of two domains: DNA-binding domain and transcription activation domain of regulatory GAL4 protein. Proteins of interest are fused with individual domains, expressed in yeast, and, if interaction occurs between those two proteins, GAL4 protein is expressed. Otherwise, those domains are not functional separately (96). As bait, however, proteins that normally function as transcription activators cannot be used unless the regions that cause activation are deleted (97). Furthermore, the identification of interacting partners can involve colony polymerase chain reaction analysis and sequencing, but that makes this method very time-consuming (98). Among the method’s main disadvantages are the inconvenience of false positive interactions (99) and absence of the possibility for posttranslational modification in yeast. That means the detection of PPIs requiring these modifications is not possible. The way to avoid this inconvenience is through co-expressing of the enzyme responsible for posttranslational modification. All investigated protein–protein interactions are localized in the yeast nucleus, and therefore it is difficult to study PPIs for membrane proteins, extracellular proteins, or toxins (93). If the two hybrid screening is the method of choice for identifying potential interacting partners for bacterial membrane proteins, then signal sequences targeting proteins to the outer membrane should be removed. Membrane proteins located in yeast nucleus probably will not be in their natural conformations (100). The two hybrid screening is usually combined with other experimental methods to confirm the identified PPIs. Clements et al. used this genetic method to reveal unknown interactions between enterohemorrhagic bacteria *E. coli* and human host (101). The two hybrid screening was applied to elucidate host–pathogen interaction of HIV-1 Vpu protein, which is involved in degradation of CD4 domain (102).

This approach was also employed in a systematic search for human hepatocellular proteins interacting with NS5A protein in the hepatitis C virus (103) and in identifying a new role of *Neisseria gonorrhoeae* outer membrane proteins in an infectious process in host epithelial cell (100).

### Biochemical approach in characterization of protein–protein interactions

#### *Cross-linking method*

The cross-linking approach is a method whereby two proteins or two regions within a protein are joined by covalent bond (104). This technique offers the possibility to stabilize transient protein–protein interactions during the cell dynamics and the possibility to identify the interaction site (105). The entire detection process is conducted in physiological conditions and *in vivo*. However, the binding strength of interaction cannot be easily determined (106). The method of protein cross-linking coupled with mass spectrometry is a new and powerful one wherein the cross-linked peptides can be identified. This method further enables detecting specific regions of interaction, such as domains, single loops, or helices (107). A statistical model has evolved for data evaluation that differentiates true interactions from false interactions. Furthermore, this method is not limited by the size of a protein complex (107). The selection of a cross-linking reagent for the experiments is the most important step during protein cross-linking that is coupled with mass spectrometry analysis (108,109). A broad spectrum of cross-linkers is available, including photo-cleavable and chemically cleavable cross-linkers (110,111). Among these are N-hydroxysuccinimide esters (112) and formaldehyde (113). A new amide bond is formed between the peptide residues (usually lysine) and the cross-linker and generates a new fragmentation pathway that differs from that of linear peptides (114). Problems could arise, however, when one wishes to determine the interaction partners for low-abundance proteins inside the cells. Under these circumstances, linear proteins overwhelm the cross-linked partners in the sample. Available solutions are based upon peptide base enrichment using strong cation exchange (115), multiple proteases for digestion (116), or cross-linking coupled with affinity purification (117–119) and with the further possibility to apply a cleavable cross-linker (120). Protein complexes are purified (by immunopurification) and followed by enzymatic digestion and mass spectrometry analysis after cross-linking reaction. Most cross-linkers interact specifically with the basic side chain of lysine residues and hinder digestion by trypsin. This results in missed cleavage sites and leads to long peptides and incomplete fragmentation, and therefore diminished sensitivity (116). To reduce these undesirable effects, multiple proteases (LysC, ArgC, AspN, and GluC) with different cleavage sites could be used or a cross-linking reagent that does not block lysines, such as formaldehyde, in order to form nonspecific cross-links (113,116).

#### *Affinity purification*

The method of affinity purification (AP) enables selective purification of proteins from a complex mixture based upon the reversible interaction between a ligand immobilized on solid support matrix and a target protein usually presented in crude cell lysate. Other compounds lacking the specific affinity will pass through the column while bounded target proteins can be readily eluted (121). For uncovering potential interaction protein partners AP can be used and then followed by mass spectrometry (122). In contrast to the two hybrid screening, this method enables characterizing PPIs while working within conditions closer to those of their physiological niches. The main advantages of AP include the possibilities it offers for characterizing multiprotein complexes (123) and for identifying posttranslational modifications (124). The target protein of interest, or bait, is fused with epitope tags on N-terminus or C-terminus. A wide range of special tags is available, such as Flag-tag, hexahistidine tag, streptavidin-binding peptide (SBP), Strep II tag, hemagglutinine (HA) tag, glutathione S-transferase (GST), calmodulin-binding peptide (CBP), and starch-binding domain (SBD). Another alternative consists of special green fluorescent protein (GFP) tags that additionally allow direct visual detection of tagged protein in the cellular environment, where they are expressed in the endogenous form (125). Moreover, multiple proteins can be labelled in a single experiment. In epitope tag selection, its characteristics such as solubility, immunogenicity, location of the epitope, size, binding, and elution conditions must always be kept in mind. So far, there is no universal epitope tag suitable for any kind of protein (126,127). There is a certain probability that tagged proteins could be folded improperly, thus altering a given protein's function or mislocalizing it within the cell (128,129). During AP, many nonspecific proteins bind to the affinity matrix. This cannot be completely eliminated, and it raises the possibility for false positive results to occur while creating a huge background. Bead proteome has been characterized to address these challenges. It contains proteins

binding nonspecifically to the affinity matrix, which may be agarose, sepharose, or magnetic beads (130). In order to distinguish the real interacting partners among many false positive results or co-purifying contaminants, AP in combination with quantitative proteomics is offered as a method of choice (131). Mass spectrometry-based quantitative proteomics approaches involve metabolic labelling (e.g., stable isotope labeling by amino acids in cell culture, or SILAC (132)), chemical labelling (e.g., isobaric tag for relative and absolute quantitation, or iTRAQ (133), and isotope-coded affinity tag, or ICAT (134)) or a label-free method (135–137) in connection with mass spectrometry. SILAC is carried out *in vivo* at the protein level and is regarded as the most accurate quantitation strategy. Labelling of samples at the beginning of an experimental procedure allow for minimizing experimental errors and sample loss (138,139). Protocols include metabolic labelling of cells, lysis of cells, mixing of lysed samples, and AP followed by mass spectrometric analysis. Two different experimental procedures for sample mixing are available: the PAM (purification after mixing) and MAP (mixing after purification) approaches. In the case of PAM, heavy (wild-type cells) and light (bait protein) cell lysates are mixed prior to AP. This allows for identification of stable PPIs and very simple sample handling. A disadvantage of this method consists in the exchange of proteins, as “light” labelled proteins bound to the bait are replaced by their “heavy” counterparts. In the MAP approach, protein purification is performed separately from equal amounts of heavy and light cell lysates. Afterwards, the eluates are mixed together. MAP enables potential identification of dynamically and stably interacting proteins, and it eliminates the problem of exchange between differentially labelled forms of proteins (140,141). Real interacting partners are discerned and characterized from a nonspecific background by the ratio of peak intensities in the mass spectrum for peptide pairs termed the abundance ratio, and this ratio should be close to 1 (142,143). Another possibility for reducing but not completely removing unspecific background is tandem AP (144,145), whereby target proteins are fused with two AP tags linked by cleavage site to allow highly selective two-stage protein enrichment (145). This prolonged purification can retain only stable interacting protein pairs while weak or transient interactions tend to be lost. Using formaldehyde cross-linking coupled with SILAC and tandem affinity purification, however, can covalently link the protein partners together and yield their successful identification (146).

This method has its own technical and biological limitations. Tagged bacterial proteins are expressed ectopically in an uninfected eukaryotic host cell. Proteins expressed out of context could lead to false positive interactions. Otherwise, changing the physiology of a cell (e.g., by infection) may influence protein expression or physiology of organelles that ultimately can affect PPI (147).

A method recently developed is known as BioID and consists in proximity-dependent labeling of proteins in eukaryotic cells. A prokaryotic biotin ligase (BirA) is fused with the bait protein and expressed in the mammalian cell, where it subsequently biotinylates the amine groups of the neighboring proteins in the presence of excess biotin in cultured media. Biotinylated proteins can then be isolated by affinity purification and identified by LC-MS analysis. BioID allows the detection of interacting and neighboring proteins in their native cellular environments (148–150).

The bacteria most studied for PPI between host and pathogen is *Salmonella*. An effort has been undertaken to explore targets of effector proteins in host cell environment while combining AP and quantitative mass spectrometry-based proteomics (151,152). Another experimental procedure combines *in vivo* cross-linking with formaldehyde, tandem AP, and LC-MS quantification (153). Quantitative proteomics using the SILAC approach has been used to help narrow down and point out potential interaction partners in host cell for bacteria *Ch. Trachomatis* (154). Another large-scale study combining AP and mass spectrometry resulted in a comprehensive network of membrane proteins of *Chlamydia trachomatis* and its interaction partners in host proteome. The study elucidated mechanisms by which this pathogen establishes its privileged intracellular niche (155).

### **Biophysical approach in characterization of protein–protein interactions**

The development of biosensors or sensor chips offer us a new method for analyzing and identifying new PPIs (156) in host–pathogen relationships, including those of bacteria vs. host cell (157–159), virus vs. host cell (160), and yeast vs. host cell (161,162). The detection methods usually connected with biosensors are the optical methods interferometry, fluorescence spectroscopy, and surface plasmon resonance (SPR) (163). Sometimes SPR is coupled with mass spectrometry, which provides information on the structures of molecules attached to a chip’s surface (164).

Distinct tags (i.e., fluorescent, affinity, or radioisotope) could be used to identify interacting proteins on a chip. Due to steric hindrance, however, tags could influence a protein's ability to interact. Considering this disadvantage, a label-free SPR method typically is employed (165). Surface-plasmon resonance is based on measurement of small changes in refractive index at the sensor metal surface (166,167). Refractive index changes are induced by the binding of analyte to the bait proteins immobilized onto the sensor's surface (163). Functional and full-length proteins or protein domains are immobilized onto a treated microscope slide. Proteins are attached onto the sensor surface, either randomly by using various chemicals or uniformly with orientation determined by different ligands (165). Limitations arise when the attached proteins are immobilized in a range of orientations or undergo partial denaturation at the chip's surface. Only when the proteins are uniformly oriented and properly folded, posttranslational modifications are present, and the attached proteins are optimally spaced will they allow protein–protein interactions (166). Moreover, the wet environment is very important for proteins during the experiment (168). Benefits of these methods include that no labels are required, sensitivity and accuracy are high, detection limits are relatively low, and repeatability is good. A disadvantage of the method is that nonspecific interaction between the sensor surface and analyte can occur, thereby interfering with measurement (163).

## **CONCLUSION AND PERSPECTIVES**

As we know, interactions between host and pathogen are always unique and differ among individual pathogenic species. Many protein–protein interactions between host cell and pathogen have been elucidated, but still many more interactions remain to be revealed. Continuing to build this knowledge will help us to understand infection processes, host cell defense mechanisms, and pathogens' offensive strategies. These observations could also lead to uncovering potential therapeutic targets within infection processes or help to develop new vaccines. Identification of host–pathogen interaction takes place in a simulated host environment. It would be interesting to monitor and identify these interactions directly upon infection by pathogen within a host cell in its physiological environment and in real time. We thereby could acquire a comprehensive picture of the infection process rather than just parts of a puzzle which must subsequently be put together to obtain a full image, as is the case today of present experiments. We strove here to outline and specify the most suitable approaches for detecting PPI. There is no single, unique best method for doing that, and the most useful approaches seem to involve combinations of different methods. Thanks to advances in computational tools for PPI prediction, it is possible to narrow the candidate lists of potentially interacting proteins. Moreover, interactive databases enable us to find all PPIs previously discovered.

## **CONSENT FOR PUBLICATION**

Not applicable.

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All authors contributed equally to the work.

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## **CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest regarding the publication of this article.

## **ADHERENCE TO ETHICAL STANDARDS**

This article does not contain any studies involving animals performed by any of the authors. This article does not contain any studies involving human participants performed by any of the authors.

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