11 Proteomics and Host–Pathogen Interactions: A Bright Future?

David G. Biron[1,], Dobrin Nedelkov[2], Dorothée Missé[3] and Philippe Holzmuller[4]*

[1]HPMCT Proteome Analysis, St-Elphège, QC, Canada, [2]Institute for Population Proteomics and Intrinsic Bioprobes, Inc., Tempe, AZ, USA, [3]GEMI, Centre IRD de Montpellier, Montpellier, France, [4]CIRAD UMR 17 Trypanosomes, Campus International de Baillarguet, Montpellier, France

11.1 Introduction

Living organisms are constantly exposed to pathogens. In any **environment**, a molecular war begins when a host encounters a pathogen. In many host–pathogen associations, the molecular war was in progress a long time ago. Nevertheless, a disease as an outcome of a pathogen attack remains an exception rather a rule. Most host species have acquired strategies by selective pressure to mislead the pathogen and to win fight during their crosstalk (i.e., molecular dialogue). However many pathogen species have acquired strategies by selective pressure to bypass the host defenses to win the molecular war and to ensure the completion of its life cycle. Pathogens remain a significant threat to any host species. Critical to the mitigation of this threat is the ability to rapidly detect, respond to, treat, and contain the pathogen transmission. For many centuries, some scientific fields (i.e., agroecology, evolutionary ecology, evolutionary medicine, biochemistry, microbiology, medicine, veterinary medicine, immunology, and molecular biology) have surveyed host–parasite interactions to improve our understanding of pathogenic diseases and to prevent pathogen transmission in host populations.

During the course of human history, pathogenic diseases have seriously affected many societies worldwide. In Europe, one of the most dramatic disease events was the great plague pandemic of the mid-fourteenth century (Watts, 1997; Achtman et al., 2004). Notably, pathogenic diseases are a leading cause of premature death in the world. Pathogenic diseases result from an intimate relationship between a host and a pathogen which involves molecular "crosstalk." Clearly, elucidation of this complex molecular dialogue between host and pathogen is desirable in order to improve our understanding of pathogen virulence, to develop pathogen-specific

* E-mail: dbiron@clermont.inra.fr

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host biomarkers, and to define novel therapeutic and vaccine targets. **Proteomics** applications to decipher host−parasite interactions are in their infancy and should lead to new insights on host **specificity** and on the evolution of pathogen virulence. In this chapter, we present the interest of proteomics to survey host−pathogen interactions, a synthetic review of previous proteomics studies, the pitfalls of the current approach in surveys, new conceptual approaches to decipher host−parasite interactions, a new avenue to decipher the crosstalk diversity involved in trophic interactions in an habitat (i.e., the **population proteomics**), and 5-year view for future prospects on proteomics and host−pathogen interactions.

11.2 Interest of Proteomics to Study Host−Pathogen Interactions

Since the start of the **genomic** era in the early 1990s, many parasitologists and molecular biologists are confident that complete sequencing of the **genome** of the partners in host−pathogen associations for pathogens with simple life cycles (i.e., one host) and in host−vector−pathogen associations for pathogens with complex life cycles (i.e., at least two hosts) will enable total understanding of the molecular mechanisms involved in most pathogenic diseases and will contribute to finding new drugs for treating them (Hochstrasser, 1998; Degrave et al., 2001). Unfortunately, little progress has been achieved in the control of such diseases as malaria and sleeping sickness, despite decades of intensive genomic projects on host−pathogen interactions, vaccines, and chemotherapeutics. Pathogens continue to be a major cause of morbidity and mortality in humans and domestic livestock, especially in developing countries (Ouma et al., 2001; Ryan, 2001; Guzman and Kouri, 2002; Gelfand and Callahan, 2003).

Until now, many parasitologists and molecular biologists have focused their studies on DNA analyses based on the central dogma of molecular biology—that is to say, the general pathway for the expression of genetic information stored in DNA. Although the basic blueprint of life is encoded in DNA, the execution of the genetic plan is carried out by the activities of proteins. The fabric of biological diversity is therefore protein-based, and natural selection acts at the protein level (Karr, 2008). At the end of the twentieth century it had become clear to many parasitologists and molecular biologists that knowing genome sequences, whilst technically mandatory, was not in itself enough to fully understand complex biological events like the immune response of a host to a pathogen infection or the molecular strategies used by pathogens to thwart the host defenses during their interaction (Barret et al., 2000; Ashton et al., 2001; Fell, 2001; Fields, 2001; Schmid-Hempel, 2008).

The evolution of any given species has tremendously increased complexity at the level of pre- (gene splicing, mRNA editing) and posttranslational (phosphorylation, glycosylation, acetylation, etc.) gene−protein interaction. The genomics era has revealed that: (1) DNA sequences may be "fundamental," but can provide little information on the dynamic processes within and between host and parasite during

their physical and molecular interaction (Barret et al., 2000; Ashton et al., 2001); (2) the correlation between the expressed "transcriptome" (i.e., total mRNA transcription pattern) and the levels of translated proteins is poor (Anderson and Seilhaver, 1997; Gygi et al., 1999; Maniatis and Tasic, 2002); and (3) a single gene can produce different protein products (Fell, 2001; Fields, 2001; Maniatis and Tasic, 2002). Moreover, the structure, function, abundance, and even the number of proteins in an organism cannot yet be predicted from the DNA sequence alone (Anderson and Anderson, 1996; Gygi et al., 1999; Barret et al., 2000). Also, posttranslational modifications such as phosphorylation and glycosylation are often extremely important for the function of many proteins, although most of these modifications cannot yet be predicted form genomic or mRNA sequences (Gygi et al., 1999). Thus, the biological phenotype of an organism is not directly related to its genotype (i.e., DNA sequences).

Epigenetic systems control and modify gene expression. Almost all the elements of epigenetic control systems are proteins (Anderson and Anderson, 1996). The cells of an organism are reactive systems in which information flows not only from genes to proteins but in the reverse direction as well (Hochstrasser, 1998). The **proteome** is the genome-operating system by which the cells of an organism react to environmental signals (Anderson and Anderson, 1996). It comprises an afferent arm, the cytosensorium (i.e., many cellular proteins are sensors, receptors, and information transfer units from environmental signals) and an efferent arm, the cytoeffectorium (i.e., in cells, reaction of the genome via regulation of either individual proteins or a group of proteins in response to environmental changes).

Proteomics is the study of the proteome. In a broad sense, the proteome (i.e., the genome-operating system) means all the proteins produced by a cell or tissue. Proteomics will contribute to bridge the gap between our understanding of genome sequence and cellular behavior. Proteomics offers an excellent way to study the reaction of the host and pathogen proteomes (i.e., genome-operating systems) during their complex biochemical crosstalk (Biron et al., 2005a,b). Using the first generation proteomics approach, two-dimensional electrophoresis (2-DE) and **mass spectrometry** (MS), posttranslational modifications of host and pathogen proteins (such as phosphorylation, glycosylation, acetylation, and methylation) in reaction to their interaction can be detected. Such modifications are vital for the correct activity of numerous proteins and are being increasingly recognized as a major mechanism in cellular regulation. Although 2-DE offers a high-quality approach for the study of host and/or pathogen proteomes, several proteomics tools have been developed that complement this approach (Gygi et al., 1999; Fung et al., 2001; Lopez and Pluksal, 2003; Wu et al., 2003; Bischoff and Luider, 2004). Table 11.1 shows a comparison of the most popular proteomics tools.

11.3 Retrospective Analysis of Previous Proteomics Studies

The host–pathogen crosstalks reflect the balance of host defenses and pathogen virulence mechanisms. Postgenomic technology promises to revolutionize many

Table 11.1 A Comparison of Proteomics Tools

Name of Technique	Separation	Quantification	Identification of Candidate Protein Spots	Hydrophic Proteins	Low Expressed Proteins	Requirement for Protein Identification	Potential for Discovering New Proteins	Detection of Specific Isoforms	Relative Assay Time	Cost to Acquire and to Use
2-DE	Electrophoresis: IEF PAGE	Densitomery of stains	Mass spectrometry (PMF;MS/MS)	Dependent on detergents used	Marginal	No	Yes	Yes	Moderate	Cheap
2-DIGE	Electrophoresis: IEF PAGE	Densitometry of Cy3- and Cy5-labeled proteins normalize to Cy2	Mass spectrometry (PMF;MS/MS)	Dependent on detergents used	Moderate (especially with scanning gels)	No	Yes	Yes	Moderate	Expensive
MuDPIT	LC−LC of peptides	None	Mass spectrometry (MS/MS)	Theoretically better than electrophoresis but not systematically examined	Moderate, often used with large sample amounts	No	Yes	Yes	Rapid	Moderate
ICAT™	LC of peptides	Through use of heavy and light tags	Mass spectrometry (MS/MS)	No better than 2-DE	Moderate	No	Yes	No	Rapid	Moderate
SELDI-TOF-MS	Binding of proteins based on their chemical and physical characteristics	Comparison of MS peaks	Requires series of samples or coupling to second MS instrument	Moderate	Marginal to moderate	No	Yes	No	Rapid	Expensive
Protein arrays	Antibody-based chips (binding to affinity reagent)	Densitometry of binding	Binding to particular affinity reagent	Unknown	Unknown	Yes	No	Yes	Rapid	Cheap

2-DE: two-dimensional electrophoresis; 2-DIGE: two-dimensional difference in gel electrophoresis; MuDPIT: multidimensional protein identification technology; LC: liquid chromatography; LC−LC: tandem liquid chromatography; PAGE: polyacrylamide gel electrophoresis; ICAT: isotopecoded affinity tags; SELDI-TOF-MS: spectrum-enhanced laser desorption ionization−time of flight−mass spectrometry; PMF: peptide mass fingerprint; MS/MS: tandem mass spectrometry.

fields in biology by providing enormous amounts of genetic data from model and nonmodel organisms. Proteomics promises to bridge the gap between our understanding of genome sequences and cellular behavior involved in host−pathogen interactions. Proteomics offers the possibility to characterize host−pathogen interactions from a global proteomic view. To date, most proteomics surveys on host−parasite interactions have focused on cataloguing protein content of pathogens and identifying virulence-associated proteins or proteomic alterations in host response to a pathogen. Also, many parasitologists and molecular biologists have used proteomics to find pathogen-specific host biomarkers for rapid pathogen detection and characterization of host−pathogen crosstalks during the infection process. In this section, a synthetic retrospective of previous proteomics studies on host−pathogen interactions and some pitfalls of these surveys are presented.

11.3.1 Deciphering of the Molecular Strategies Involved in Parasite Immune Evasion

To elude the vigilance of the immune system of a host, particularly a mammal, a causative microorganism must actually act as a double agent. Indeed, the broad immunity has a natural or innate and adaptive component. Innate immunity constitutes the first antimicrobial defense and rapidly induces soluble mediators such as complement, inflammatory cytokines, and chemokines together with effector cells such as macrophages and natural killers, in order to control or delay the spreading of the infectious agent. Then a specific response of adaptive immunity will take place to eliminate pathogens that would have survived innate immune response (Roitt and Delves, 2001). These immune selective pressures have conducted pathogens to develop mechanisms to modulate and alter host responses or to evade phagocytosis. As a result of these host pathogen interactions, protein expression profiles of the host immune system (susceptibility/tolerance factors: antibodies, cell receptors, biochemical pathway, …) and of the pathogen (virulence/pathogenicity factors: **antigens**, immunomodulators) are mutually modified (Zhang et al., 2005; Coiras et al., 2008; Holzmuller et al., 2008).

Depending on the pathogen type (virus, bacteria, fungi, unicellular or multicellular parasites), strategies of interactions will be different and the subversion of the host immune responses will exhibit specificities at the protein level (for reviews, see Walduck et al., 2004; Biron et al., 2005a; Viswanathan and Früh, 2007). In fact, these molecular dialogues and conflicts can be seen as a chess game between the host immune cell populations and the pathogen populations, in which the pathogen plays with the whites (i.e., it starts the game). Because of differences in host−pathogen organisms' size and ratio, leading to size differences of respective proteomes, the pathogen proteome could be considered as overwhelmed by the host proteome during the interactions. But in terms of immune evasion, this is not limiting because the immune system works on a qualitative basis, which constitutes a second advantage for the pathogen that can induce large-scale damages with low amounts of molecules. By contrast, this represents one major limitation to characterize host−pathogen interactions, but also a challenging perspective for

proteomics technology. This is why retrospectively proteomics studies were mainly conducted to evidence pathogenic virulence and pathogenicity factors (Ouellette et al., 2003; Texier et al., 2005; Sims and Hyde, 2006; Van Hellemond et al., 2007; Liu et al., 2008a,b; Bird and Opperman, 2009; Jagusztyn-Krynicka et al., 2009; Premsler et al., 2009; Weiss et al., 2009; Steuart, 2010; Bhavsar et al., 2010; Holzmuller et al., 2010).

Independently of the proteomics workflow used for analysis, parasite immune evasion could be illustrated by at least three strategies that are commonly widespread among pathogens: (1) immune evasion based on antigenic variation, (2) inhibition of adaptive immunity activation systems, and (3) host mimicry. In African trypanosomes, the antigenic variation of the variant surface glycoprotein (VSG) constituting the surface coat of the parasite is well described (Morrison et al., 2009). But as in proteomics study, the parasite population, which has switched the VSG, is so poorly represented it goes undetected, and therefore always keeps one step ahead of host immune responses. Also, in trypanosomatids, *Leishmania amastigotes*, which establish within macrophage (a major immune effector cell), developed the ability to degrade class II **major histocompatibility complex** molecules to prevent Th1-type immunity to be induced (Antoine et al., 2004). This strategy can be likened to the concept of histocompatibility testing in the case of transplant to avoid rejection of non-self by the recipient. Another protozoan parasite, *Toxoplasma gondii*, generates its parasitophorous vacuole with elements of the plasma membrane from the targeted host cells, thus using the host "self" to evade immune recognition (Plattner and Soldati-Favre, 2008). These few examples perfectly illustrate how difficult it is to decipher, at the protein level during interactions, the pathogen molecular components involved in immune evasion. Nevertheless, advances in proteomics offer challenging perspectives to decipher the molecular war in host−pathogen interactions.

11.3.2 Host Proteome Responses to Parasite Infection

While it seems obvious to say that when a pathogen will infect a host, it will react by expressing molecules that can be characterized by clinical proteomics, it is surprising how few studies are devoted to this research. Yet the discovery for biomarkers differentiating an infected state from a healthy state is the heart of the Infectious Disease Research (te Pas and Claes, 2004; Azad et al., 2006), and expression proteomics has quickly developed to characterize the differential expression of proteins encoded by a particular gene and their posttranslational modifications in biological fluids and tissues (Fournier et al., 2008; Hood et al., 2009; Wilm, 2009). In characterizing the host proteome responses to a pathogen infection, different levels of analysis have to be considered: soluble biomarkers expressed in biological fluids (e.g., serum, saliva, urine, and cerebrospinal fluid), tissue biomarkers indicative of an organ response and cellular biomarkers indicative of a cell-type response (e.g., immune cells).

Interestingly, the majority of the proteomics studies on host response to infection were performed on viral deregulation of host cells proteome ex-vivo (Liu et al.,

2008a,b; Sun et al., 2008; Zheng et al., 2008; Antrobus et al., 2009; Lee et al., 2009; Pastorino et al., 2009; Vester et al., 2009; Zhang et al., 2009, 2010a,b). These works allowed characterization at the molecular level, the overall modifications in protein profiles of the target cells, and were of high interest to the better understanding of the pathogen influence on its host. In bacteria, studies have evaluated the mode of action of known toxins or bacterial components on host cells (Kuhn, et al., 2006; Shui et al., 2009). Concerning parasites, ex-vivo experiments on host cell−parasite interactions have highlighted molecular details of manipulation strategies suffered by target cells during toxoplasmosis Chagas disease or malaria (Teixeira et al., 2006; Nelson et al., 2008; Wu et al., 2009b). Curiously, few works directly focused on the subversion of the immune system, mainly through monocyte/macrophage deregulation (Oura et al., 2006; Fischer et al., 2007).

As a paradox, the most striking studies on host proteome response to parasite infection were performed on arthropod (infectious diseases vectors)−parasite interactions, probably because the parasite induced a strong phenotype modification (Biron et al., 2005c; Rachinsky et al., 2007), particularly in the case of insect behavior manipulation (Lefèvre et al., 2007a,b). Although few in number, taken together these pioneering analyses of the response of the proteome of the host to a pathogen paved the way for the dynamic analysis of host−pathogen interactions. These approaches deserve to be strengthened and extended to all infectious diseases to increase and improve knowledge of the molecular dialogue and conflict that govern host−pathogen interactions.

On the other hand, the clinical aspect is important in infectious diseases, and a number of studies have sought to characterize more comprehensively the proteome response of the host to infection in biological fluids, with a purpose diagnosis. One interesting pioneering study was performed in rabbits and allowed detection of intra-amniotic infection by proteomic-based amniotic fluid analysis (Klein et al., 2005). For human diseases or those of livestock, the biological fluid, which should enable the detection of infection in the host serum linked to host proteome response. Several studies performed on this biological sample have allowed discriminating host−commensal from host−pathogen interactions in *Candida albicans* (Pitarch et al., 2009) and determining the immunome of pathogens (Sakolvaree et al., 2007; Ju et al., 2009). Moreover, in African trypanosomiasis, proteomics analysis of the serum not only was indicative of the host response to infection, but also was promising for characterizing disease progression toward neurological disorder (Papadopoulos et al., 2004; Agranoff et al., 2005). This illustrates how proteomics will help in considering at different analytical levels the host proteome response to a pathogen infection, with the prospect of benefits in improving diagnostic and therapeutic.

11.3.3 Biomarkers Linked to Infection Process by a Pathogen Using SELDI-TOF-MS Technology

High-throughput proteomic technology offers promise for the discovery of disease biomarkers and has extended our ability to unravel proteomes. In this section,

we will focus on the surface-enhanced laser desorption time-of-flight mass spectrometry (SELDI-TOF-MS) technology. This mass spectrometric-based method requires a minimal amount of sample for analysis and allows the rapid high-throughput analysis of complex protein samples (De Bock et al., 2010). SELDI-TOF-MS differs from conventional matrix-assisted laser desorption ionization (MALDI)-TOF-MS because the target surfaces to which the proteins and matrices are applied are coated with various chemically active ProteinChip® surfaces (ion exchange, immobilized metal affinity capture, and reverse phase arrays). Therefore, it is possible to fractionate proteins within a mixture, or particular classes of proteins, on the array surface prior to analysis. As with MALDI, different matrices can be used to facilitate the ionization and desorption of proteins from the SELDI array surface (Merchant and Weinberger, 2000).

This technology was initially applied to the discovery of early diagnostic or prognostic biomarkers of cancer (Petricoin and Liotta, 2004; Xiao et al., 2005; Yang et al., 2005). Recently, this technology has been used to discover fluid or tissue protein biomarkers for infectious diseases such as HIV-1 (Luo et al., 2003; Sun et al., 2004; Missé et al., 2007; Luciano-Montalvo et al., 2008; Toro-Nieves et al., 2009; Wiederin et al., 2008), hepatitis B and C viruses (Poon et al., 2005; Kanmura et al., 2007; Fujita et al., 2008; Molina et al., 2008; Wu et al., 2009a,b), severe acute respiratory syndrome (Pang et al., 2006) and BK virus (Jahnukainen et al., 2006), African Trypanosomiasis (Stiles et al., 2004; Agranoff et al., 2005), infection of Artemia by cestodes (Sánchez et al., 2009), tuberculosis (Liu et al., 2010), bacterial endocarditis (Fenollar et al., 2006), and *Helicobacter pylori* infection (Das et al., 2005).

Certain individuals are resistant to HIV-1 infection, despite repeated exposure to the virus. The analysis of resistance to HIV infection is one of a research avenue which has the hope of resulting in the development of a more effective treatment or a successful preventive vaccine against HIV infection. However, the molecular mechanism underlying resistance in repeatedly HIV-1-exposed, uninfected individuals is unclear. A complementary transcriptome and SELDI-TOF-MS analyses on peripheral blood T cells, and plasma or serum from EU, their HIV-1- infected sexual partners, and healthy controls have been performed (Missé et al., 2007). This study detected a specific biomarker associated with innate host resistance to HIV infection, as an 8.6-kDa A-SAA cleavage product.

In the same vein, understanding the virus−host interactions that lead to patients with acute Hepatitis C virus (HCV) infection to viral clearance is a key toward the development of more effective treatment and prevention strategies. SELDI-TOF-MS technology have been used to compare, at a proteomic level, plasma samples respectively from donors who had resolved their HCV infection after seroconversion, from donors with chronic HCV infection and from unexposed healthy donors (Molina et al., 2008). A candidate marker of about 9.4 kDa was found to be higher in donors with HCV clearance than in donors with chronic infection. This biomarker was identified by nanoLC-Q-TOF-MS/MS as Apolipoprotein C-III and validated by Western Blot analysis.

11.4 Pitfalls of the Current Conceptual Approach in many "Parasito-Proteomics" Studies

During the postgenomics era, the "**parasito-proteomics**" have been suggested to study host–pathogen interactions. The "parasito-proteomics" is the study of the reaction of the host and parasite genomes through the expression of the host and parasite proteomes during their biochemical crosstalk. Studies in "parasito-proteomics" are performed either by following the expression of the parasite proteome during infection by a given parasite (Langley et al., 1987; Moura and Visvesvara, 2001; Cohen et al., 2002; Boonmee et al., 2003), by the reaction of the host proteome following an invasion by a parasite species (Moskalyk et al., 1996; Thiel and Bruchhaus, 2001; Cohen et al., 2002), or by the injection of immune **elicitors** (Han et al., 1999; Vierstraete et al., 2004).

Some elegant studies on the proteome responses of insect hosts during their **molecular crosstalk** with pathogens concluded that insects could rapidly react to infection by a given pathogen (i.e., bacteria or fungi) by producing immune-induced proteins including peptide or polypeptides (Hoffman, 1995). However, a key point is to define whether the host genomic responses elicited through activation of immune constitutive proteins, induction, and/or suppression of proteins during the infection by a parasite represent a nonspecific response that might be induced by any pathogen. Many "parasite-proteomics" studies dealt with a limited framework by deciphering the host proteome response during the infection process by a specific pathogen (Huang et al., 2002).

The classical approach in parasito-proteomics makes it possible to identify proteins of interest for a given host–parasite association. For example, Wattam and Christensen (1992) associated some polypeptides with the genome response of the host mosquito, *Aedes aegypti* (Diptera: Culicidae), with the invasion of the filarial worm, *Brugia malayi*. This pioneering study provided important new information on the response of the host insect to invasion by a specific parasite species. Nevertheless, it was *not* possible to determine whether that the response detected in *Ae. aegypti* is specific to *B. malayi* (Spiruria: Filariidae), or whether it can be observed for any parasitic worm species invading a dipterous host.

Other studies have revealed the limitations of the current approach in parasito-proteomics by showing that in the host–parasite interaction, many immune mechanisms are involved (constitutive, induced, specific, or otherwise) (Haab et al., 2001; Levy et al., 2004; Vierstraete et al., 2004). By using two treatments, the injection of lipopolysaccharides (LPS) and a sterile injury, Vierstraete et al. (2004) were able to disentangle proteome modifications induced by immunity from those induced by a physical stress. Levy et al. (2004) studied the immune response of the fruitfly *Drosophila* to bacterial (*Micrococcus luteus* and *Escherichia coli*) and fungal (*Beauveria bassiana*) infections. The data revealed that 70 of the 160 protein spots detected were differentially expressed at least fivefold after a bacterial or fungal challenge. In addition, the majority of these spots were specifically regulated by one pathogen, whereas only a few spots corresponded to proteins altered

in all cases of infection. In summary, the classical approach in parasito-proteomics has many benefits in terms of understanding fundamental aspects of gene−protein functional interactions. Unfortunately, it is not applicable to different pathogen species (and as such, does not encourage the growth of knowledge of general host proteome responses), nor does it necessarily help in the creation of a proteomic database with a holistic relevance to the understanding of host−parasite interactions.

Moreover one classical pitfall in "parasite-proteomics" surveys is the use of a single proteomic technique. Recently, Bridges et al. (2008) have demonstrated by using a battery of proteomic techniques to characterize the plasma membrane subproteome of bloodstream form of *T. brucei* that these techniques are complementary since each one has identified a unique subset of proteins of the plasma membrane. Although 2-DE offers a high-quality approach for studying the host and/or parasite proteomes, several proteomic tools have been developed that will complement this traditional proteomic tool (see Table 11.1). In the same way, MS analysis could take advantages of combined techniques. For example, the widely used analysis of peptides via collisionally activated dissociation (CAD) is rapid and results in reproducible predictable fragmentation behavior for a given peptide sequence, a substantial proportion of peptide product ion mass spectra does not result in successful sequence identification (Steen and Mann, 2004). This is well illustrated by Hart et al. (2009), who showed that a substantial number of proteins from trypanosome flagellum were identified in their three independent flagellar proteome investigations, but also that combining electron transfer dissociation (ETD) with CAD allowed the identification of 168 proteins that were not recognized in their first analysis. This strengthens the idea of integrating both approaches and technologies to reach exhaustive protein datasets from a given biological compartment.

Finally, although 2-DE and MS have been very successfully employed to identify proteins involved in host−parasite crosstalks, many recent papers have emphasized the pitfalls of 2-DE experiments, especially in relation to experimental design, poor statistical treatment, and the high rate of "false positive" results with regard to protein identification (Barret et al., 2005; Biron et al., 2006a; Holzmuller et al., 2008). It is necessary to be careful in the interpretation of results for the previous "parasito-proteomics" surveys on host−parasite interactions (see Biron et al., 2006a; Holzmuller et al., 2008).

11.5 Toward New Conceptual Approaches to Decipher the Host−Parasite Interactions for Parasites with Short or Complex Life Cycle

One main goal of "parasite-proteomics" surveys is to find proteins for use as pathogen-specific host biomarkers and to decipher host−pathogen crosstalks. Some

recent papers emphasize that a significant number of surveys were done with a nonrigorous experimental design and without a conceptual approach to disentangle a general host proteome response from a specific host proteome response during the interaction with a pathogen (Tastet et al., 1999; Ashton et al., 2001; Huang et al., 2002; Biron et al., 2005a; Holzmuller et al., 2008, 2010). A new attitude is essential to improve the reliability of proteomics data on host−pathogen interactions. Lately, some conceptual approaches have been proposed to researchers working on host−pathogen interactions to improve the reliability of "parasite-proteomics" results and to stimulate the creation of proteomic database with a holistic view of host−pathogen interactions. Thus, in this section, three new avenues to decipher host−pathogen interactions for any pathogen species (i.e., with simple or complex life cycle) are presented.

11.5.1 A Holistic Approach to Disentangle the Host and Parasite Genome Responses During Their Interactions

Some proteomics studies have shown common features in the innate response of plants, insects, and mammals (Broekaert et al., 1995; Rock et al., 1998; Cao et al., 2001; Taylor et al., 2003). The plant defense response is mediated by disease resistance genes (R genes), which are abundant throughout the genome and confer resistance to many microorganisms, nematodes, and/or insects (Dixon et al., 2000). R genes of several families of plants studied to date show homology with the Drosophila receptor Toll and the mammalian interleukin-1 receptor (Rock et al., 1998). In addition, plants, invertebrates, and vertebrates produce the "**defensins**" class of peptides, which are pathogen-inducible (Broekaert et al., 1995; Hoffman, 1995). Some peptides and/or proteins used by phytophagous or animal parasites to modify the genome expression of their host, share many structural and functional homologies. Thus, for example, phytoparasitic root-knot nematodes of the genus *Meloidogyne* secrete substances into their plant hosts in order to make a giant cell used as a feeding site (Abad et al., 2003; Doyle and Lambert, 2003). A similar system is observed for the zooparasite *Trichinella spiralis* (Stichosomida: Trichinellidae) (Jasmer, 1993). Furthermore, the injection of a peptide isolated from nematode secretions to either plant protoplasts or human cells enhances cell division (Goverse et al., 2000). The mechanism is not yet well-known but protein induction is considered as a strong possibility.

Currently, many data are obtained by genomic and proteomics projects concerned with host−parasite interactions. Nevertheless, as mentioned earlier, generally little effort is made to elaborate such projects with respect to a holistic view of the goal to increase knowledge concerning immune responses of a host along with the biochemical crosstalk between host and pathogen/parasite. Thus far, parasito-proteomics studies are in their infancy but have already led to new insights concerning molecular pathogenesis and microorganism identification (Moura et al., 2003; Vierstraete et al., 2004; Levy et al., 2004; Biron et al., 2005d). However, many parasito-proteomics studies have been done with powerful tools but without

a conceptual approach to disentangle the host and parasite genome responses during their interactions.

A new holistic approach proposed to parasitologists and molecular biologists based on evolutionary concepts of the immune response of a host to an invading parasite (for more details, see Biron et al., 2005a). For instance, this new conceptual approach enables the classification of the host genomic response to infection by a parasite according to the immune mechanisms used (constitutive versus induced) and the degree of specificity. From an evolutionary-ecological point of view, host immune responses to a particular parasite can be plotted on a chart according to the immune mechanisms used (constitutive versus induced) and degree of specificity. The first axis of the defense chart refers to the immune mechanisms employed by the host with the two extreme cases: (1) a constitutive immune mechanism used by the host to rapidly impair the invasion by a parasite; and (2) an induced immune mechanism which has the advantage of avoiding a costly defense system, yet meanwhile has the disadvantage that the parasite might escape host control (Schmid-Hempel and Ebert, 2003). The second axis of the defense chart refers to the degree of specificity of the host immune response.

Whatever the tactics used and the degree of specificity, the host genome ensures the adequate operation of the immune response via the proteome (genome-operating system). For each immune tactic, many proteins are implicated. Consequently, any researcher in parasito-proteomics working with the immune defense chart will be able to categorize the host genome reaction for any given parasite at any given time. Also, for the pathogen, from an evolutionary-ecological point of view, parasite molecular strategies used to counteract host immune system can be plotted on a chart according to the infection mechanisms used (constitutive versus induced) and degree of specificity. This type of approach should be as much hypothesis generating for parasito-proteomics as for evolutionary ecology itself.

Lately, pioneer proteomics studies on parasite-induced alteration of host behavior (widespread transmission strategy among pathogens) have been carried out on six arthropod host−parasite associations: two orthoptera−hairworm associations, two insect vector−pathogen associations, and two gammarid−parasite associations (Lefèvre et al. 2009). These parasito-proteomics studies were based on the conceptual approach suggested by Biron et al. (2005a,b). Thus, in each study, many biological treatments have been effected to control the potential confusion resulting from proteins that are nonspecific to the manipulative process and to find the protein potentially linked with host behavioral changes. Also, for each study, to limit the possible effects of multiple infection and/or host sex-specific factors on the host proteome response, only mono-infected host males were used for the protoemics analysis. These parasito-proteomics surveys on the parasitic manipulation hypothesis showed that proteomic tools and the conceptual approach suggested by Biron et al. (2005a,b) are sensitive enough to disentangle host proteome alterations and also the parasite proteome alterations linked to many factors such as the circadian cycle, parasitic status, parasitic emergence, quality of a habitat, and manipulative process.

11.5.2 Pathogeno-proteomics: A "New" Avenue to Decipher Host−Vector−Pathogen Interactions

Relationships between pathogens and their hosts and vectors depend on a molecular dialogue being tightly regulated. The reciprocal influence of a pathogen with its host or vector will affect the level of their genomes and their expression, respectively (Holzmuller et al., 2008). Variability and cross-regulation increase from genomic DNA (mutations, rearrangement, methylation) through RNA transcripts (initiation, splicing, maturation, editing, stability) to functional proteins (initiation, folding, posttranslational modifications, localization, function). Pathogeno-proteomics is a new approach to decipher host−vector−pathogen interactions, which integrate modifications at all analytical levels (genome, transcriptome, proteome: whole cell content, and secretome: naturally excreted−secreted molecules) through the analysis of their end-products' profile (Figure 11.1).

The concept is based on management with drawers of the analytic workflow, from the determination of number of experimental treatments and design of the biological material preparation to the dedicated proteomics and **bioinformatics** tools needed to answer a research question in cell immunobiology (directly involved in host−pathogen interactions) but also in ecology and evolution, population's biology and adaptive processes (Biron et al., 2006b; Holzmuller et al., 2008; Karr, 2008). Moreover, it has been proved that the results of this type of integrated approach has a concrete impact on the discovery of the causes of infectious diseases, as well as on improving the diagnosis, vaccine development, and rational drug design (Doytchinova et al., 2003; Bansal, 2005; Chautard et al., 2009). Despite a theoretical aspect (Kint et al., 2010), the pathogeno-proteomics concept brought new insights into important aspects of cell signaling (Kleppe et al., 2006) and molecular medicine (Ahram and Petricoin, 2008; Ostrowski and Wyrwicz, 2009). As an example, proteomics and bioinformatics tools enable the formulation of relevant biological

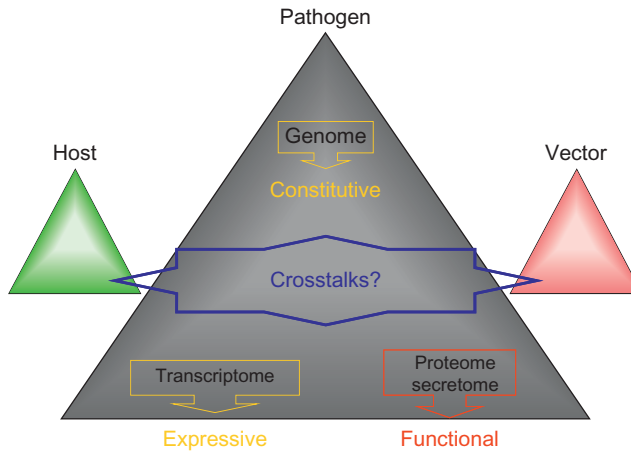

Figure 11.1 Pathogeno-proteomics: integrating analytical levels in host−vector−pathogen interactions.

hypothesis on why part of the fungal population is killed while a significantly high percentage survives in *C. albicans*−macrophage interactions (Diez-Orejas and Fernández-Arenas, 2008), leading to edition of a specific database for studying *C. albicans*−host interactions (Vialás et al., 2009). Direct applications in terms of discovery of antifungal drug targets or design of new effective antibacterial vaccines become reality (Tournu et al., 2005; Jagusztyn-Krynicka et al., 2009). Other studies have also highlighted the pathogenic changes in the brain of SIV-infected monkeys (Pendyala et al., 2009), adaptive metabolic changes in *Trypanosoma cruzi* and *Trypanosoma congolense* (Grébaut et al., 2009; Roberts et al., 2009) or molecular biomarkers of intestinal disorder induced by *H. pylori* or *Tritrichomonas muris* (Wu et al., 2008; Kashiwagi, et al., 2009). More recently, the use of model organisms interacting with infectious agent of medical importance emphasized the complexity and pathogen-specificity of the worm's immune response (Bogaerts et al., 2010). Taken together these examples demonstrate the potential of the concept of pathogeno-proteomics and promote this new research avenue.

11.5.3 Host−Pathogen Interactomes

The past few years have witnessed the birth of new biological entities named interactomes. They correspond in an "ideal world" to the complete set of protein−protein interactions existing between all the proteins of an organism. In reality they are far from complete since an unknown number of interactions are yet to be discovered. Current interactomes are only a part of the whole set of possible interactions occurring within an organism or between organisms. They are generally assembled from the results of large-scale two hybrid screens (LS-Y2H) around 6000, 4000, 23,000, and 5500 interactions for yeast (Uetz et al., 2000; Ito et al., 2001), the nematode, *Caenorhabditis elegans* (Maupas) (Li et al., 2004), *Drosophila* spp. (Giot et al., 2003; Formstecher et al., 2005), and humans (Rual et al., 2005, Stelzl et al., 2005), respectively; and the interactions identified by low-scale experiments described in the literature that may be eventually compiled in specialized databases (e.g., INTACT [Hermjakob et al., 2004], MINT [Zanzoni et al., 2002], HPRD [Peri et al., 2003], BIND [Alfarano et al., 2005]). Consequently, they do not reflect temporal influences because interactions are gathered from different cell types, tissues, development stages, and types of experiment.

Interactomes form large intricate networks leading to a renewed vision of cell biology as an integrated system. However, extracting and revealing the functional information they contain depends on our ability to analyze them in detail. For this, bioinformatics methods that partition the interaction network into functional modules have been proposed. These modules usually correspond to group of proteins involved in the same pathway, the same protein complex, or the same cellular process. Since interaction networks are represented by complex graphs in which nodes correspond to proteins and edges to the interactions, a number of these network analysis methods have been grounded on principles that derive from graph theory.

Noticeably, a functional module or a class of protein that is functionally related and based on network analysis can be deduced from: (i) a search for graph regions particularly densely populated by interactions (Bader and Hogue, 2003; Spirin and

Mirny, 2003); (ii) the similarity between the shortest paths in the graph (Rives and Galitski, 2003); (iii) the progressive disconnection of the graph using a calculation of edge "betweenness" (Girvan and Newman, 2002; Dunn et al., 2005); and (iv) the sharing of interactors (Brun et al., 2003; Samanta and Liang, 2003) or a combination thereof (Brun et al., 2004). Some of these methods use the functional annotations of the protein (such as Gene Ontology annotations) to annotate the functional modules they predict. Based on the characteristics of a protein of unknown function to some of these annotated modules or classes, a putative function for such proteins can be proposed (Brun et al., 2003). Currently, specialized methods are being developed to investigate the interactomes. But it is clear that the field starts to move forward. Thus some softwares, plug-ins, or servers are available for free use by the proteomics research community (for instance, MCODE in Cytoscape [Shannon et al., 2003]; Prodistin [Baudot et al., 2006]).

Although the deciphering of the interactomes of the main model organisms is not yet complete, studies of the interactomes of pathogens are increasing. The first pathogens to be investigated in terms of their interactomes were the hepatitis C virus (Flajolet et al., 2000) and the bacterium, *H. pylori* (Rain et al., 2001). More recently, the interactomes of the herpes viruses (Uetz et al., 2006) and the malaria parasite, *Plasmodium falciparum* (LaCount et al., 2005), have been determined. This makes one believe that in the near future, as initiated by Uetz et al. (2006), the docking of the interactomes of pathogens onto those of their hosts will be possible. The analysis of the host−pathogen interactome (i.e., "docked interactomes") during the host−pathogen crosstalk is certainly a very promising and exciting aspect of interactomics because of its obvious potential impacts on human and animal health (Figure 11.2). The host−pathogen interactome will permit to identify host and pathogen protein networks linked to specific functions during their interaction.

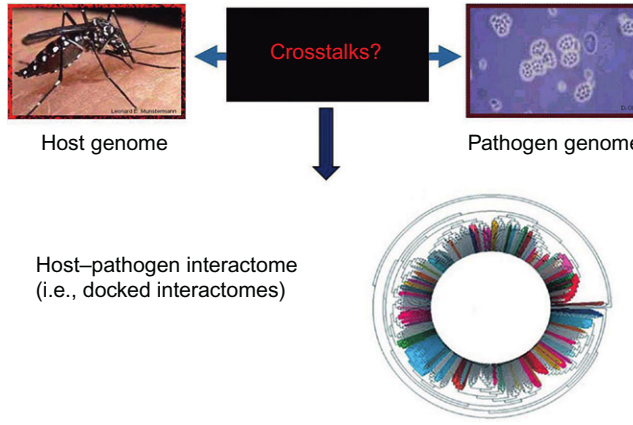

Figure 11.2 A new biological entities named host−pathogen interactome corresponding to complete set of protein−protein interactions existing between all the proteins of a host and a pathogen during their interaction.

11.6 Population Proteomics, an Emerging Discipline, to Study Host–Parasite Interactions

The host susceptibility to a pathogen and/or the pathogen virulence are often fluctuating within a host population even when infected hosts are collected in the same habitat and at the same time. This host phenotypic variability can be caused by three factors: (i) host genotype and/or pathogen genotype, (ii) different environmental experiences (habitat fragmented in microclimates), and (iii) host coinfection by pathogens (competition or mutualism among co-infecting pathogens within hosts). What are the host–pathogen crosstalks at individual and population scales in a habitat? Is it possible to detect and to decipher the host proteome variability within a habitat for the molecular mechanisms and for the protein networks involved in the host–pathogen interactions? In this section, a new emerging discipline in proteomics, the population proteomics, and its prospects are presented with results of some pioneer studies on this topic, especially in human population proteomics.

11.6.1 Prospects with Population Proteomics for Any Living Organisms

One limiting factor for the first generation of proteomics tools (such as 2-DE) is the amount of proteins required to study the host and/or pathogen proteome expression(s) during their interactions. Most surveys in "parasite-proteomics" were done by pooling many individuals for any treatment (such as infected and noninfected hosts) required to answer to a query. Thus, with this kind of experimental protocol, no data can be acquired on the interindividual variation in expression of host and pathogen proteomes during their crosstalk. New proteomics tools and methods have been developed as 2D-LC/MS that can permit to study the interindividual variation of molecular crosstalk in host–pathogen associations (Nedelkov et al., 2004; Predel et al., 2004; Brand et al., 2005).

At beginning of the century, Dobrin Nedelkov proposed a new scientific field in proteomics: the population proteomics (Nedelkov et al., 2004). Population proteomics was defined as the study of protein diversity in human populations, or more specifically, targeted investigation of human proteins across and within populations to define and understand protein diversity with the main aim to discover disease-specific protein modulations (Nedelkov, 2008). Biron et al. (2006b) have proposed to broaden the "population proteomics" concept to all living organisms with the aim to complement **population genetics** and to offer a new avenue to decipher the crosstalk diversity involved in trophic interactions in a habitat, since the execution of the genetic plan is carried out by the activities of proteins and natural selection acts at the protein level (Karr, 2008; Cieslak and Ribera, 2009).

The apparent separation between genomics and proteomics that leads to different perspectives on the same ecological reality is a fundamental limitation that needs to be overcome if complex processes, like adaptation, pathogen virulence, and host susceptibility are to be understood. Population proteomics coupled with

population genetics has a great potential to resolve issues specific to the ecology, the evolution of natural populations, the dynamic of host susceptibility to pathogens, the evolution of pathogen virulence, and the range of host genotypes that can be infected with a given pathogen genotype in host−parasite interactions. Some perspectives for the population proteomics are resumed in Figure 11.3. Even if we are yet far from this "promised land", a better understanding of the information contained in proteomics markers should permit an impressive amount of information to be gathered on the past as well as current environmental conditions experienced by a given population of a species, something that could be summarized as "show me your proteome and I will tell you who you are, where you are from, and where you should go from here."

Lately, pioneer surveys on population proteomics have been carried out with classical proteomic tools (i.e., 2-DE and MS) to determine the genetic variability between species and between populations of a given species (Chevalier et al., 2004; Diz and Skibinski, 2007; Valcu et al., 2008), to identify biochemical signatures linked to particular habitat and/or environmental conditions (Thiellement et al., 1999; Pedersen et al., 2010) and phylogenetic studies (Navas and Albar 2004, Dorus et al., 2006). Nedelkov et al. (2005, 2007) have investigated the human plasma proteins' diversity using approaches similar to enzyme-linked immunosorbent assay but utilizing MS as method of detection (Nedelkov, 2008). These pioneer results should help to discover disease-specific protein modulations but also to find pathogen-specific protein biomarkers.

The next subsection will present more in details the Nedelkov results on protein diversity in human populations.

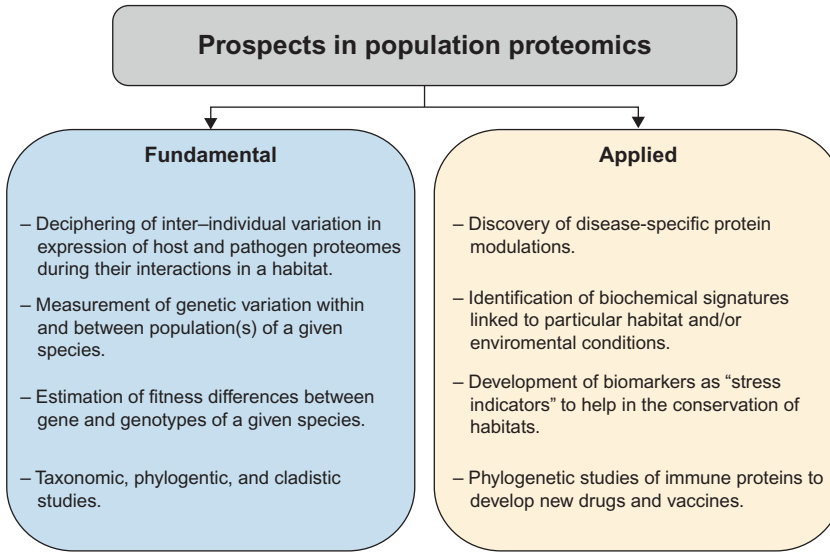

Figure 11.3 Potential of population proteomics as an emerging discipline in proteomics.

11.6.2 Human Population Proteomics

Human population proteomics deciphers protein diversity in human populations. In broader terms, human population proteomics can be compared to human population genomics, where individuals are interrogated with the aim of cataloguing common genetic variants and determining how they are distributed among people within populations and among populations in different parts of the world (Nedelkov et al., 2004, 2006; Nedelkov, 2005). Although human population proteomics cannot (yet) claim such outreach and goals, it has the potential to become an important proteomics subdiscipline as the tools and approaches that enable it become more embraced and practiced.

Human population proteomics does not engage the study of entire proteomes because it is very likely that, for a specific cell or tissue proteome, there is no definitive set and number of proteins that is common to all within a group or a larger population. Instead, human population proteomics focuses on interrogation of a selected number of proteins but from a large number of individuals, to delineate the distribution of specific protein modifications within these subpopulations. Hence, targeted protein analysis approaches utilizing MS as detection method are employed. MS measures a unique feature of each fully expressed protein—its molecular mass. Changes in the protein structure resulting from structural modifications are reflected in its molecular mass and can be detected via MS, without a priori knowledge of the modification. The MS methods utilized in human population proteomics must be capable of analyzing hundreds if not thousands of samples per day, with high reproducibility and sensitivity. Hence, top-down MS approaches utilizing affinity ligands are the most likely methods of choice for population proteomics (Nedelkov, 2006). Surface-immobilized ligands can be utilized to affinity to retrieve a protein of interest from a biological sample, after which the protein (with or without the affinity ligand) is introduced in a mass spectrometer. One of the first affinity MS methods developed was mass spectrometric immunoassay (MSIA) (Nelson et al., 1995). The approach combines targeted protein affinity extraction with rigorous characterization using MALDI-TOF MS (Figure 11.4). Protein(s) are extracted from a biological sample with the help of affinity pipettes derivatized with polyclonal antibodies. The proteins are eluted from the affinity pipettes with a MALDI matrix, and are MS-analyzed. Enzymatic digestion, if needed, is performed on the MALDI target itself. Specificity and sensitivity, as in traditional immunoassays, are dictated by the affinity-capture reagents—the antibodies.

However, a second measure of specificity is incorporated in the resulting mass spectra, wherein each protein registers at specific *m/z* value. During data analysis, the major signal in the mass spectrum that corresponds to the targeted protein is initially evaluated; it should be within a reasonable range (e.g., error of measurement of $<0.05\%$) from the value of the empirically calculated mass obtained from the sequence of the protein deposited in the Swiss-Prot databank. Once this mass value is confirmed (or observed to be shifted), the presence of protein modifications is noted by the appearance of other signals in the mass spectra (usually in the vicinity of the native protein peaks), or by mass shifts of the major protein signal.

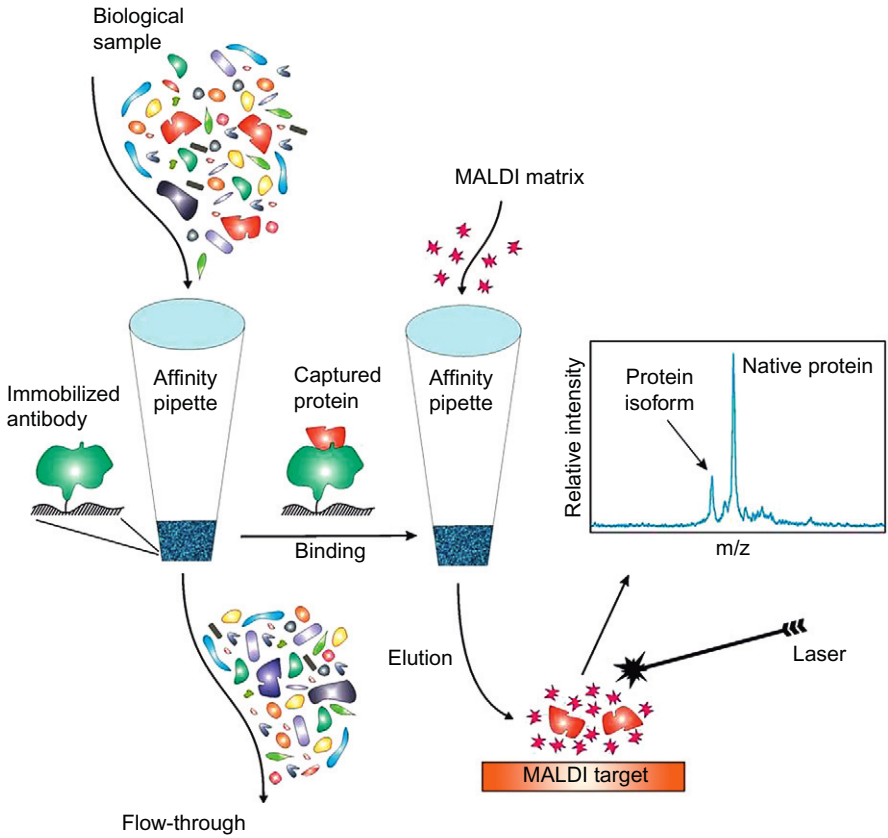

Figure 11.4 Schematics of the MSIA approach.

Modifications can be tentatively assigned by accurate measurement of the observed mass shifts (from the wild-type protein signals and/or in-silico calculated mass) and knowledge of the protein sequence and possible modifications. The identity of the modifications is then verified using proteolytic digestion and mass mapping approaches in combination with high-performance MS.

In an initial study of human protein diversity using mass spectrometric methods of detection, 25 plasma proteins from a cohort of 96 healthy individuals were investigated via MSIA (Nedelkov et al., 2005). The protocol and an example of the data generated for one of the protein, transthyretin (TTR), are outlined in Figure 11.5.

The TTR MSIA were performed in parallel on the 96 human plasma samples using affinity pipettes derivatized with anti-TTR antibody. Following mass spectrometric analysis, data matrix containing all tentatively assigned modifications was assembled. Then, peptide-mapping experiments were performed on selected number of samples to identify the specific modifications and finalize the modifications database. The data for all 25 proteins is presented in Figure 11.6, which lists the

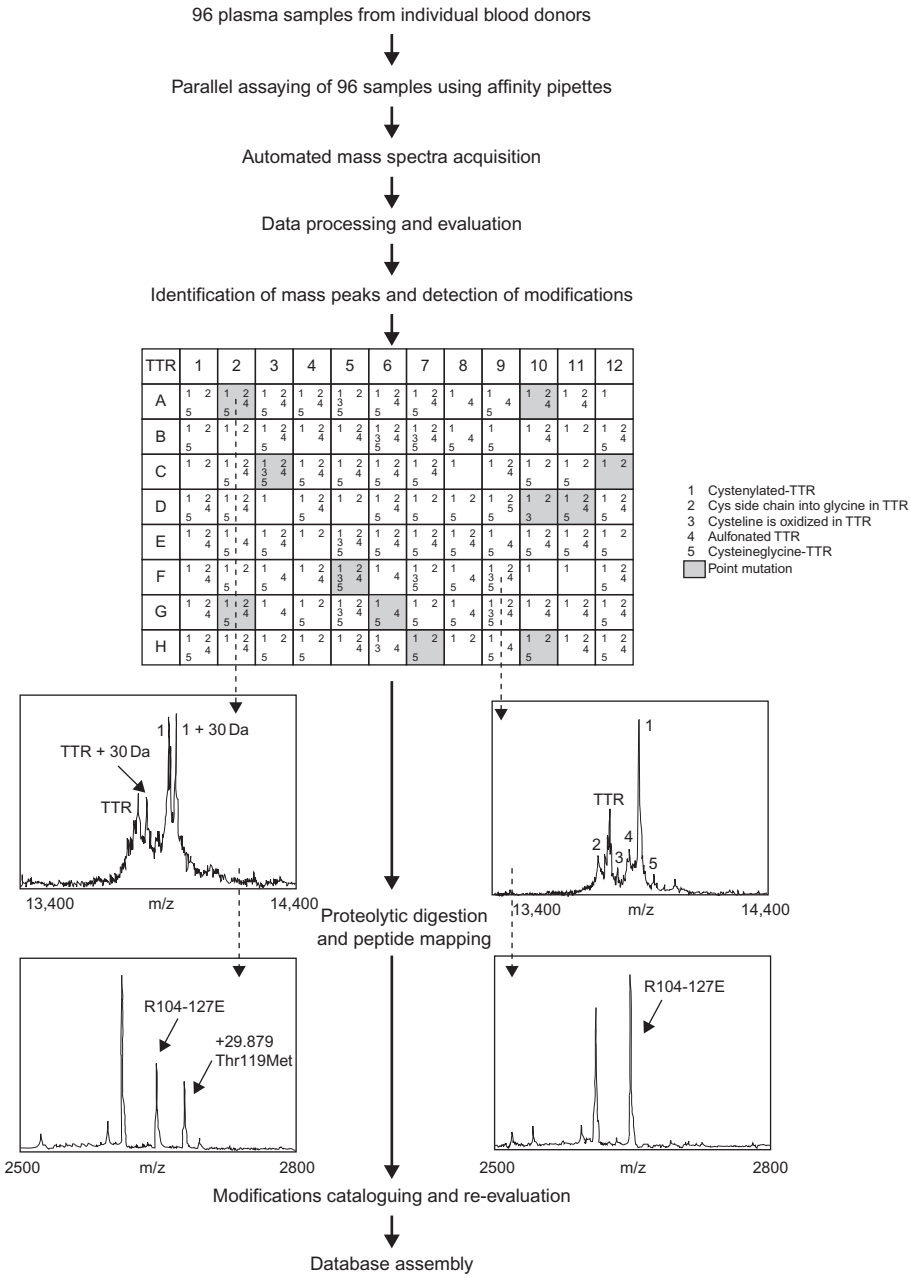

Figure 11.5 An outline of a population proteomics approach using TTR as an example. m/z: mass-to-charge ratio; TTR: transthyretin.

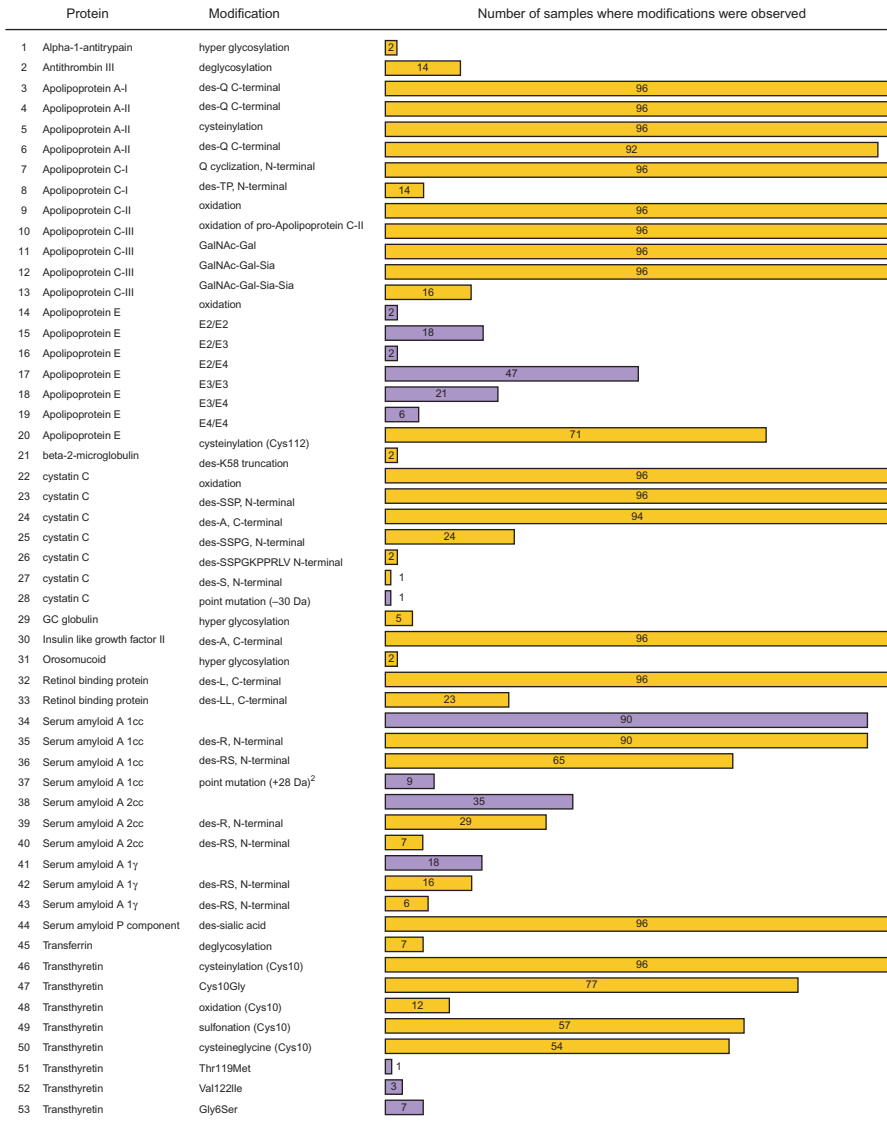

Figure 11.6 Modifications observed in 18 of the 25 proteins analyzed from 96 human plasma samples.

modifications observed for 18 of the 25 proteins studied (modifications were not observed for 7 proteins), and shows the frequency of each modification in the 96-samples cohort. A total of 53 protein variants were observed for these 18 proteins, stemming from posttranslational modifications and point mutations.

The largest number of posttranslationally modified protein variants was found to be C- or N-terminal truncated protein isoforms. Deglycosylation, oxidation, and

cysteinylation were also observed among several of the proteins. Among the point mutations detected for four of the proteins, notable was the high incidence of point mutations for apolipoprotein E and TTR, which is consistent with genomic studies that have found these proteins to be highly polymorphic. The overall frequency of the modifications in the 96-sample cohort was wide ranged. Fourteen modifications were observed in all 96 samples, suggesting that they must be regarded as wild-type protein forms. Others, such as most of the point mutations, were present in only few of the samples. Overall, 23 of the modifications were observed in more than 65% of the samples, and 20 in less than 15% of the 96 samples analyzed. Upon further data analysis, and taking into the consideration the gender, age, and ethnicity of the individuals who provided the samples, it was determined that the Gly6Ser mutation in TTR was detected only in individuals of Caucasian origin, which is consistent with existing knowledge about the occurrence of this common nonamyloidogenic population polymorphism in Caucasians (Connors et al., 2003). Another correlation was observed in regard to interprotein variations in specific individuals: all seven individuals for which carbohydrate deficient transferrin was detected were also characterized with deglycosylated antithrombin III.

Following this small scale protein diversity study, a second study of human protein diversity was recently carried out wherein the number of samples was greatly expanded in order to get an accurate view of the distribution of some of the protein modifications in the general population (Nedelkov et al., 2007). One thousand individuals from four geographical regions in the USA (California, Florida, Tennessee, and Texas) were selected and the protein modifications for beta-2-microglobulin (b2m), cystatin C (cysC), retinol binding protein (RBP), transferrin (TRFE), and TTR were delineated (in the 96-sample study, these five proteins accounted for 19 of the 53 protein variants observed). The results of the study are summarized in Figure 11.7, which lists the protein modifications observed and the frequency of each in the 1000-samples cohort.

A total of 27 protein modifications (20 posttranslational modifications and 7 point mutations) were detected, with various frequencies in the cohort of samples. Variants resulting from oxidation were observed most frequently, along with single amino acid truncations. Least frequent were variants arising from point mutations and extensive sequence truncations. In total, six modifications were observed with high frequency (present in $>80\%$ of the samples), five were of medium frequency ($20-50\%$ of the samples), and sixteen were low frequency modifications observed in $<7\%$ of the samples. Nine of the low frequency modifications were not observed in the 96 individuals studied. Thus, by increasing the size of the population, it became possible to detect these low-occurrence protein modifications. When the frequencies of the modifications in the two studies were compared, an excellent correlation was obtained. For example, in both cohorts $\sim 7\%$ of the individuals were characterized with carbohydrate deficient transferrin. Upon further data analysis based on the gender, age, and geographical origin of the individuals who provided the samples, it was determined that the samples obtained from California contained significantly less protein modifications than the samples obtained from Florida, Tennessee, and Texas, even though the samples from all four states were

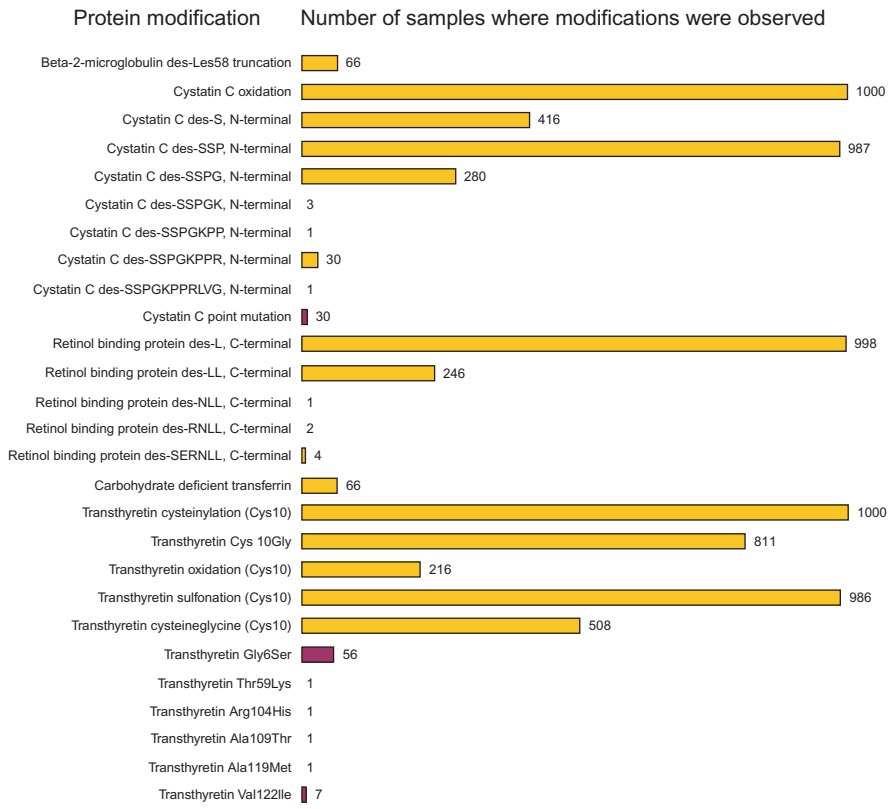

Figure 11.7 Modifications observed for five proteins studied from 1000 human plasma samples.

collected in the same way within a 3-month window in the spring of 2005, and stored under identical conditions until analysis. Correlations were also made in regard to the gender distribution of two protein modifications. Carbohydrate deficient transferrin was observed in ∼1% of the females and ∼10% of the males in the 1000-samples cohort. Carbohydrate deficient transferrin is an FDA-approved clinical biomarker for alcoholism, and this gender correlation can partially be explained by the higher prevalence of alcohol dependence in males than in females. The second gender correlation was related to cystatin C: all 10 of the cystatin C point mutations were found in males.

Two conclusions can be made from these two systematic studies of protein modifications and variants. First, MS is capable of detecting structural protein modifications, and, when coupled to immunoaffinity separations, it can be employed in a high-throughput systematic study of human protein diversity. Second, the human protein diversity is far more complex than the variation observed at the genetic level. While it might be premature to declare the human proteins variation "the next big thing," it is reasonable to predict that assessing human proteome variations

among and within populations will be a paramount effort that can facilitate biomarker discovery. Such an endeavor would represent a paradigm shift in proteomics with significant clinical and diagnostic implications, as protein variations, quantitative and qualitative, begin to be associated with specific diseases.

11.7 5-Year View

11.7.1 Metabolomics, a Key "Omics" Tools to Decipher Host–Parasite Crosstalks

Metabolomics (i.e., metabolic profiling) is concerned with the measurement of global sets of low-molecular-weight metabolites to detect changes in cell behavior and organ function. The term "metabolome" refers to the complete set of metabolites found in an organism (Peltonen and McKusick, 2001). Metabolomics approaches use high-throughput analytical techniques such as chromatography, NMR spectrometry, and MS to measure populations of low-molecular-weight metabolites in biological samples. These large-scale efforts involve the identification and quantification of known and unknown metabolites in host tissues and fluids. Metabolite profiles can be important indicators of pathological states of a host and raise the possibility of identifying novel markers linked to the infection process of a specific pathogen.

Pathogens, especially extracellular pathogens with a complex life cycle, such as malaria and sleeping sickness, must constantly monitor and respond to environmental changes in their intermediate (i.e., insect vector) and final (human and/or animal) hosts. How pathogens detect these changes is a black box, but they must have the ability to sample changes in nutrients and other small molecules. Afterward pathogens reprogram their gene expression profile in response to host environmental changes. The metabolomics is likely to bridge data from other "omics" tools. Correlation between the pathogen metabolites expressed during its life cycle in its hosts with the global view of genome and proteome expression profiles may lead to new insight into how a pathogen interacts with host cells during a host–vector–pathogen crosstalk.

11.7.2 New Diagnostic Tools and Identification of New Therapeutic Targets

Despite the efforts of recent years, we still lack reliable biomarkers for diagnosis, prediction of clinical outcomes for many infectious diseases, and therapeutic follow-up of human diseases. Specific proteomic fingerprints might be present in biological fluids or tissues in response to the infection and could be useful for early detection of the disease, by noninvasive (saliva, urine, serum) or invasive (cerebrospinal fluid, tissue biopsies) sampling, or could constitute therapeutic targets. Before identifying of a biomarker having great potential to become an important diagnostic tool, it is important to verify the clinical applicability of a technique.

In fact, different criteria are necessary for a clinically applicable technique: reproducibility, sensitivity, specificity, and rapidity.

Over the past few years, several studies have demonstrated that comparative protein profiling using a newly developed, high-throughput technique, without a priori knowledge of the proteins present, namely SELDI-TOF-MS, breaks new ground in diagnostics (Fenollar et al., 2006; Buhimschi et al., 2007; Kanmura et al., 2007; Wu et al., 2009a,b). This technique is a potentially powerful investigative tool to improve the understanding as well as the diagnostic/prognostic capabilities for many human diseases. Nevertheless, if using such an approach, it is important that potentially new biomarkers for early diagnosis are validated in a larger number of samples to avoid the risk of false-significant results.

Inspired by genome exploration, a metaproteomic strategy, namely proteomics shotgun, has also emerged to facilitate and accelerate the discovery of novel protein biomarkers with potential diagnostic and therapeutic potential (Swanson and Washburn, 2005; Spivey, 2009). Based on different MS workflows (e.g., capillary isotachophoresis[CITP]-based multidimensional or multidimensional LC separation systems coupled tandem MS), it promotes integration of proteomics and metabolomics datasets from direct analysis of a tissue or a biological fluid (Dowell et al., 2008; Pan et al., 2008; Fang et al., 2009; Kim and Moon, 2009; Mangé et al., 2009; Maccarrone et al., 2010). Although strategic, these technologies offer a "naïve" global approach of potential biomarkers by taking into accounts the level of expression, the posttranslational modifications, and the maturation state (Ahrné et al., 2010; Park and Yates, 2010; Zhang et al., 2010a,b). These technologies are in their infancy in the analysis of host−pathogen interactions, but some pioneering studies have already highlighted some new biomarkers with diagnostic and therapeutic potential (Florens et al., 2004; Athanasiadou et al., 2008; Lal et al., 2009; Walters and Mobley, 2009; Vaezzadeh et al., 2010).

Understanding the three-dimensional structure of proteins—their posttranslational modifications, their biological functions, and the interaction between host and pathogen molecular constituents—is necessary to validate a potential biomarker in its natural molecular environment. Advances in proteomics can consider a more functional understanding of molecular dialogue and conflict that governs host−pathogen interactions, and thus develop more efficient tools to improve diagnosis, and drug design to improve therapy of infectious diseases.

11.7.3 Bioterrorism and Proteomics

Following the events of September 11, 2001, and the subsequent postal anthrax attacks in the USA, the possibility of further bioterrorism attacks became all too real. As a direct consequence of this, the US government expanded its biodefense program, with studies ranging from basic research to applications in detection, prevention, and treatment of diseases caused by such microbiological agents. The net result of this has been great progress in understanding their genomics (Fauci et al., 2005). Efforts were focused on the three major categories of critical biological agents classified by the Centers for Disease Control and Prevention (http://www.bt.

cdc.gov/agent/agentlist-category.asp#catdef). In the postgenomic era, the benefit of having the full sequence of the genomes of these agents is obvious. There are now genome sequences for a few isolates of each species, which have made studies of comparative genomics a reality and led to important discoveries, including the diversity of closely related isolates and the identification of new putative virulence genes (Fraser, 2004). In this way, breakthrough transcriptomics and proteomics studies promise further exciting results and surprises over the next few years, which hopefully will have highly beneficial applications in terms of combating the scourge of global bioterrorism (see Morse 2007).

11.7.4 Environment and Host–Parasite Interactions

For host–pathogen interactions, the main assumption is that, over ecological time-scales, host susceptibility and pathogen virulence are fixed at the onset of the crosstalk (Bull, 1994; Dieckmann et al., 2002). Also, environmental factors are traditionally viewed as "setting the scene" for the crosstalk rather than having any explicit role once it is underway. As a result, the effect of extrinsic factors on host susceptibility and pathogen virulence during a crosstalk has received little attention. However, it is common to find in populations of a pathogen species a substantial variation in the virulence, even when pathogens are collected in the same environment and at the same time. When a biological characteristic such as the virulence is variable for both genetic and environmental reasons, two individuals may differ because they differ in genotype, because they have had different environmental experiences, or both (Elliot et al., 2002). Unfortunately, the extent to which different individual pathogens and pathogen ecotypes display different virulence abilities is poorly documented and deciphered.

Life-history traits of hosts and pathogens are shaped by coevolution processes (Wolinska and King, 2009). Infections measured under laboratory conditions have shown that the environment in which hosts and pathogens interact may affect the range of host genotypes that can be infected with a given pathogen genotype in host pathogen associations (i.e. the **specificity of selection**). Despite this important fact, environmental fluctuations are often excluded in surveys on host–pathogen interactions. Since most host–pathogen interactions are in heterogeneous environments, there is a crucial need to take into account environmental conditions in proteomics surveys. The population proteomics would be a promising prospect to resolve interesting issues specific to host–pathogen crosstalks in a varying environment (Figure 11.8). This kind of survey would bring pioneer molecular data to decipher the **reaction norm** of a genotype and to understand why pathogens sometimes evolve in a given environment toward high virulence and hosts toward high resistance. Also, these surveys would permit to assess the fixity or not of host–parasite interactomes involved in a host–pathogen association in a varying environment.

11.7.5 Human Population Proteomics

While healthy population protein diversity cataloguing is a pretty straightforward proposition, the ultimate question is how human population proteomics can enable

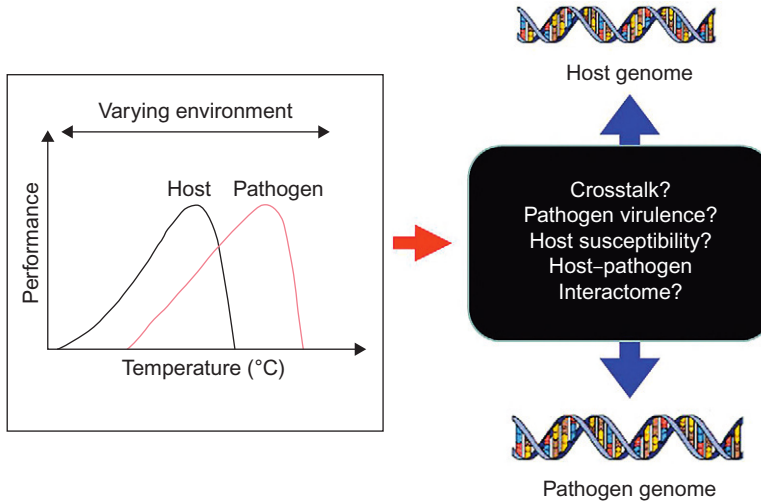

Figure 11.8 Host–pathogen interactions in a varying environment.

better disease diagnosis and management. Because of the one-protein-at-a-time approach, most studies involving cohorts of sick versus healthy control samples must first make an "educated guess" what proteins to analyze via population proteomics. To start with, there is plenty of existing data on specific plasma proteins and their quantitative modulations with specific diseases. Hence, these proteins would be the first to benefit from qualitative reassessment via human population proteomics; next in line are proteins that are within the biomarker proteins' interaction network and pathways. To illustrate this point, structural isoforms have recently been discovered for some well-established biomarkers such as cardiac troponin I (Jaffe and Van Eyk, 2006; Peronnet et al., 2006) and B-type natriuretic peptide (Brandt et al., 2006; Lam et al., 2007). These structural variants might prove to be better sensitivity and specificity biomarkers than the native proteins themselves. To assess their modulation with disease, quantitative assessments of the isoforms abundance can be made by comparing the ratios of the isoforms and the native protein signals, or via standard curve approaches. The MSIA present straightforward means of looking into the protein microheterogeneity using the well-established methods of immunoaffinity separation and mass spectrometric detection. As such, it is expected that MS-based immunoassays will readily be accepted into the clinical and diagnostic laboratories to study the effects of protein modifications in pathological processes and evaluate their potential as new biomarkers of disease.

11.8 Conclusion

From the dawn of human evolution to the influenza and HIV/**AIDS** pandemics of the twentieth and early twenty-first centuries, infectious diseases have continued to

emerge and re-emerge with great ferocity and by so doing, seriously affect populations as well as challenge our abilities to fight the responsible agents. Over the past decade, strains of many common pathogens have continued to develop resistance to the drugs that once were effective against them. In the battle against pathogens, humankind has created new megatechnologies such as massive sequencing, proteomics, and bioinformatics, but without conceptual approaches based on the evolutionary concepts. Parasite genome sequences do not themselves provide a full explanation of the biology of an organism or on the molecular war involved in host−pathogen associations. Since the 1990s, proteomic tools have been successfully employed in a large number of studies to find and identify proteins involved in biological phenomena, such as host−parasite interactions. Even so, many studies have, as outlined earlier, revealed pitfalls in the approaches used. Thus, whatever the new technological advancements, it is apparent that parasitologists and molecular biologists should attempt to improve their experimental design. This new attitude will surely improve the reliability of the data deriving from proteomics studies and will open the way for an enhanced comprehension of many biological mechanisms. In this chapter, new ways based on evolutionary concepts are suggested to enable further elucidation of the molecular complexities of host−pathogen genome interactions. These new ways could help to increase the knowledge about the molecular war involved in host−pathogen associations, taking into account the environmental factors.

Glossary

AIDS acronym for acquired immune deficiency syndrome, the gravest of the sexually transmitted diseases, or STDs. It is caused by the human immunodeficiency virus (HIV), now known to be a retrovirus, an agent first identified in 1983. HIV is transmitted in body fluids, mainly blood and genital secretions.

Antigen substances that are foreign to the body and cause the production of antibodies.

Bioinformatics the use of mathematical and informational techniques, including statistics, to solve biological problems, usually by creating or using computer programs, mathematical models, or both. One of the main areas of bioinformatics is the data mining and analysis of the data gathered by the various genome projects. Other areas are sequence alignment, protein structure prediction, systems biology, protein−protein interactions, and virtual evolution.

Defensin a substance with natural antibiotic effects found in human blood cells. There are three types of defensins. Other animal species have similar substances.

Elicitors molecules produced by the host (or pathogen) that induce a response from the pathogen (or host).

Environment elements of a habitat. In this text, "environment" refers to a broad range of biotic and abiotic conditions, including interactions with other species.

Genomics the study of an organism's genome and the use of the genes. It deals with the systematic use of genome information, associated with other data, to provide answers in biology, medicine, and industry.

Genome the full complement of genes carried by a single (haploid) set of chromosomes. The term may be applied to the genetic information carried by an individual or to the range of genes found in a given species.

Major histocompatibility complex two classes of molecules on cell surfaces. MHC class I molecules exist on all cells and hold and present foreign antigens to CD8 cytotoxic T lymphocytes if the cell is infected by a virus or other microbe. MHC class II molecules are the billboards of the immune system. Peptides derived from foreign proteins are inserted into the MHC's binding groove and displayed on the surface of antigen-presenting cells. These peptides are then recognized by T lymphocytes so that the immune system is alerted to the presence of foreign material. See Histocompatibility Testing.

Mass spectrometry a technique for separating ions by their mass-to-charge (*m/z*) ratios.

Molecular crosstalk molecular communications in a host–parasite system during their interaction.

Parasito-proteomics the study of the reaction of the host and parasite genomes through the expression of the host and parasite proteomes (genome-operating systems) during their biochemical crosstalk.

Pathogenicity the capability of a pathogen to cause disease.

Population genetics the study of the distribution of genes (the units of genetic inheritance) and genotypes (the genetic complement at one or more loci), and the mechanisms determining genetic variability within a population.

Population proteomics the study of protein diversity in populations of any species; or more specifically, targeted investigation of proteins across and within populations of a species to define and understand protein diversity and to facilitate the discovery of disease- or pathogen-specific protein modulation.

Proteome the term proteome was first used in 1995 and has been applied to several different types of biological systems. A cellular proteome is the collection of proteins found in a particular cell type under a particular set of environmental conditions, such as exposure to hormone stimulation. It can also be useful to consider an organism's complete proteome. The complete proteome for an organism can be conceptualized as the complete set of proteins from all of the various cellular proteomes. This is very roughly the protein equivalent of the genome. The term "proteome" has also been used to refer to the collection of proteins in certain subcellular biological systems. For example, all of the proteins in a virus can be called a viral proteome.

Proteomics the large-scale study of proteins, particularly their structures and functions. This term was coined to make an analogy with genomics, and is often viewed as the "next step," but proteomics is much more complicated than genomics.

Reaction norm the set of phenotypes that can be produced by a genotype under various environmental settings.

Specificity an alternative concept to explain why hosts vary in their susceptibility to parasites is that host–parasite interactions have some degree of specificity.

Specificity of selection the range of host genotypes that can be infected with a given parasite genotype in host–parasite interactions.

Transcriptome the whole set of mRNA species in one or a population of cells.

Transcriptomics Techniques to identify mRNA from actively transcribed genes.

Two-dimensional gel electrophoresis proteomics, the study of the proteome, has largely been practiced through the separation of proteins by two-dimensional gel electrophoresis. In the first dimension, proteins are separated by isoelectric focusing (separation of proteins according to their isoelectric point in a pH gradient gel), resolved on the basis of charge. In the second dimension, they are separated by molecular weight using

SDS-PAGE. To visualize the proteins, the gel is dyed with Coomassie Blue, silver, or other reagents. Spots on the gel are proteins that have migrated to specific locations.

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