Chapter 6
Host–Pathogen Interactions

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Abstract The ability to genome sequence mycobacteria and host organisms has enabled a range of system-wide approaches to be developed to explore the interplay between host and pathogen. These global analyses offer an unbiased means of generating new hypotheses to further understand bacterial pathogenesis and immune activation states. *Mycobacterium tuberculosis* high-throughput mutant screening has identified key genes and pathways involved in mycobacterial physiology or pathogenicity that are required *in vivo* or during macrophage infection. Reciprocal genome-wide RNAi-based screening approaches have highlighted host genes that play crucial roles in the immune and metabolic crosstalk with infecting bacilli. In addition to these loss-of-function screens, transcriptional profiling of the pathogen, of the host, or of both together has provided clues into the divergent metabolic states and key signalling events that characterise *M. tuberculosis* infection. Such global analyses, linked in a systems approach through interaction databases and network mapping, allow descriptive and predictive models of infection and disease to be constructed. In this chapter we review the recent developments and applications of these system-wide approaches to better understand the interactions of *M. tuberculosis* with its host.

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1 Introduction

In the post-genomic era, the development of a variety of system-wide approaches has allowed host–pathogen interactions to be examined on a global level. Genomic analyses offer an unbiased means of generating new hypotheses to further understand bacterial pathogenesis. In the case of the tuberculosis bacillus, several high-throughput *Mycobacterium tuberculosis* mutant screening studies performed during macrophage infection or in vivo have identified key genes and pathways involved in mycobacterial physiology and required for virulence. More recently, genome-wide RNAi-based screening approaches have highlighted host genes that play crucial roles in the immune and metabolic crosstalk with infecting bacilli. In addition, global gene expression profiling of the pathogen, of the host, or of both together has provided clues into the divergent metabolic states and key signalling events that characterise *M. tuberculosis* pathogenesis. As such, temporal analyses describing the changing interplay between bacilli and macrophage as infection progresses are particularly useful, allowing descriptive and predictive models to be constructed. In this chapter we review the recent developments and applications of these system-wide approaches to better understand the interactions of *M. tuberculosis* with its host. We illustrate how transcriptome analysis coupled to models of signalling and transcription networks can help to suggest novel interactions of potential importance during infection. This systems approach to interpreting host–mycobacterial interplay is summarised in Fig. 6.1.

2 Functional Genomics to Identify Mycobacterial Virulence Genes

Understanding how a pathogen and its host adapt to each other during the course of infection is key to developing new tools and better strategies to combat infectious disease. Over 10 years ago sequencing the *M. tuberculosis* genome [1], together with the development of genetic tools to inactivate genes in random or targeted approaches [2], allowed novel virulence genes and loci involved in pathogenesis and host parasitism to be discovered on a genome-wide level. Two studies published in 1999 made use of a functional genomics approach developed earlier in *Salmonella* [3], signature transposon-tagged mutagenesis (STM), using medium-size pools of *M. tuberculosis* mutants to identify *M. tuberculosis* genes important during infection in the mouse model [4, 5]. Both studies highlighted phthiocerol dimycocerosates, complex lipids of the mycobacterial cell wall, as key components of mycobacterial pathogenicity. PDIMs now constitute a prototypic example of a complex molecule of the mycobacterial cell envelope involved in pathogenesis; yet their exact function and mode of action still remain to be fully understood [6, 7]. A few years later, the generation of novel tools for transposition and tracking of transposon mutants using a microarray-based strategy, termed transposon site hybridization, allowed gene insertion events to reach saturation levels. This enabled the authors to classify virtually all mycobacterial genes required for

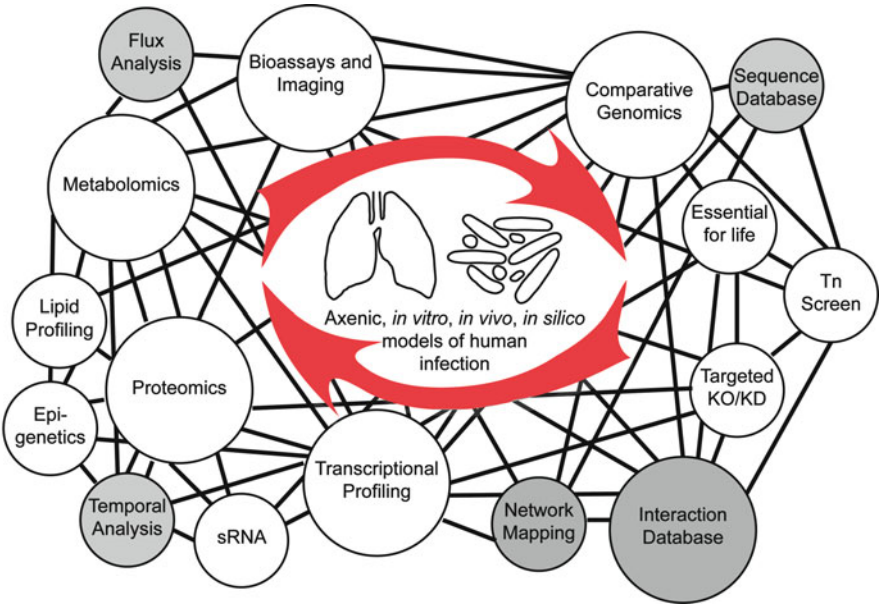

Fig. 6.1 An interaction network of techniques and approaches used to study host–pathogen interplay. The complementary methodologies are linked together by bioinformatics tools and databases (*shaded grey*) in a systems approach to understanding infection and disease

successful infection in the mouse model in a high-throughput and system-wide manner [8–10]. In these studies, Sassetti and Rubin used a library of 100,000 transposon mutants, in which almost all non-essential genes were inactivated and looked for mutants impaired in their ability to grow in various *in vitro* and *in vivo* conditions, including murine lungs. Although the mouse model is not ideal for studying mycobacterial virulence, it nevertheless provides an indication of the relative importance of mycobacterial pathways for in vivo survival. In this way, a number of genes predicted to be involved in secretion, lipid metabolism, carbohydrate transport and metabolism, inorganic ion transport and metabolism, cell envelope biogenesis, and amino acid transport and metabolism were recognised. Many of these genes had not previously been shown to play a part in mycobacterial virulence in vivo. A number of genes with unknown function specific to mycobacteria were also discovered; this raises the intriguing question of the role of ancient horizontal gene transfer events in mycobacterial physiology and pathogenicity (see below) [11]. Similar approaches have been used in other animal models that more closely resemble human disease, such as non-human primates (NHPs) [12]. Again in this study, a number of previously underappreciated genes, for example, involved in lipid metabolism and transport, biosynthesis of the cell wall, and sterol metabolism were classified to be functionally significant for mycobacterial pathogenicity in vivo.

More recently, screening approaches have been developed to detect mycobacterial genes involved in pathogenic processes at the host cell or sub-cellular levels.

M. tuberculosis genes mediating parasitism of the macrophage, the primary host cell for pathogenic mycobacteria in the lungs, have been identified through STM [13]. *M. tuberculosis* genes involved in phagosome remodelling have been determined using magnetic organelle sorting, flow cytometry and high-throughput confocal microscopy-based approaches [14–16]. The ability of pathogenic mycobacteria, such as *M. tuberculosis*, to arrest phagosome maturation and to remain in an immature, mildly acidic and non-proteolytic environment is thought to be a key feature of mycobacterial virulence [17]. Using an elegant and innovative approach based on magnetic organelle sorting from ferritin-loaded macrophages, Pethe et al. [15] isolated and characterised mycobacterial mutants defective in phagosome maturation arrest and thus trafficked to ferritin-loaded phago-lysosomes [15]. Interestingly, some of these mutants were again impaired in PDIMs synthesis or export, shedding new light on the role of these complex lipid moieties in intracellular mycobacterial trafficking, as recently reported by others [6]. In a similar approach, Stewart et al. [16] used flow cytometry to sort mycobacterial phagosomes from fluorescent LysoTracker-loaded phago-lysosomes, and was able to isolate and identify mycobacterial mutants defective in inhibition of phagosome acidification [16]. More recently, we have used high-throughput confocal microscopy to screen a genome-covering library of *M. tuberculosis* W-Beijing mutants [14]. Over 11,000 random transposon mutants were used to infect human macrophages in high-density 384-well plates in a one-well one-mutant manner. Infected cells were stained with the acid-specific dye LysoTracker. Mutants that colocalised with the dye were selected and their transposon insertion sites were sequenced. Two independent mutants in *Rv1503c* and *Rv1506c*, which belong to the same genetic locus in the mycobacterial chromosome, and two other mutants in *moaC1* and *moaD1*, which belong to another locus likely involved in synthesis of the molybdopterin cofactor, were isolated. Furthermore, we showed that the *Rv1503c/6c* locus is involved in the synthesis of trehalose-containing glycolipids, thus establishing a link between these lipids and the ability of pathogenic mycobacteria to prevent phagosome acidification. These studies illustrate how system-wide functional genomics approaches help to identify mycobacterial virulence genes and gene clusters in an unbiased manner. Strikingly, all these studies reported mutants in intergenic regions of the mycobacterial chromosome. This raises the intriguing question of the functional significance of non-coding small RNAs (sRNA) in *M. tuberculosis* pathogenicity [18]. As in other bacterial species, it is likely that sRNA play key roles in *M. tuberculosis* virulence by regulating the expression of other genes. Such findings lay the foundations for functional epigenomics in mycobacteria which will benefit from the development of new genomics tools in the future.

3 *In Silico* Mycobacterial Genomics

As more mycobacterial genomes have been sequenced over the years, genome comparison and in silico genomics have provided clues to mycobacterial pathogenicity. Comparative genomics identified the attenuation of the vaccine strain, *M. bovis*

BCG, to be a major deletion in its chromosome (the RD1 region of difference) as compared to the *M. tuberculosis* or the *M. bovis* chromosomes [19]. More recently, we and others have used *in silico* and comparative genomics to identify several chromosomal regions in *M. tuberculosis* that were most likely acquired by the ancestor of the *M. tuberculosis* complex through horizontal gene exchanges [20–23]. Strikingly, most of these regions are predicted to be acquired from environmental bacterial species, thus strengthening the long-thought hypothesis that the ancestor of *M. tuberculosis* was an environmental species that has gradually been "educated" to become pathogenic, and in particular to parasitise phagocytes [11]. Studying the role of these ancient horizontally acquired genes in mycobacterial physiology and virulence is now ongoing in several laboratories.

4 Functional Genomics to Recognise Host Genes Mediating the Response to Mycobacteria

A system-wide exploration of the role of host immuno-regulatory pathways in mycobacteria interactions is now possible because of the development of genetic tools to silence gene expression in eukaryotes using RNA interference (RNAi). Such approaches have been successfully used to identify host genes contributing to mycobacterial growth restriction in drosophila cells [24, 25] and more recently in mammalian cells [26, 27]. The future use of RNAi-based genetic screening techniques in multicellular organisms, such as the zebra fish, that can be infected by *Mycobacterium marinum*, a close relative of *M. tuberculosis*, will undoubtedly allow further understanding of the importance of specific host genes in immunity to mycobacteria in vivo. Thus, the application of whole genome approaches screening for mycobacterial survival or observable changes in macrophage–mycobacteria interactions, such as differential phagosome trafficking, has identified both host and pathogen genes that influence the outcome of infection. Comparative genomics have provided historical and geographic context to these genes and enabled mycobacterial pathogenicity to be directly associated with particular gene clusters. The transcriptional regulation of host and *M. tuberculosis* genes during infection provides yet another key perspective into these multi-factorial interactions.

5 Transcriptional Profiling Mycobacteria Interactions with Phagocytes

Techniques that exploit the differential regulation of genes during infection have been employed for many years to define the dialogue between *M. tuberculosis* bacilli and host immune cells. Selective approaches such as subtractive hybridisation [28, 29], promoter trap library screening [30], *in situ* hybridisation [31], and

quantitative RT-PCR [32] have identified key genes highlighting pathways involved in the phagocytosis and survival of *M. tuberculosis* in host cells. Sequencing of the *M. tuberculosis* H37Rv genome [1], and subsequent mouse [33] and human genomes [34], heralded the age of genome-wide expression profiling using microarrays, qRT-PCR panels or more recently RNAseq [35]. These whole genome approaches together with the continued development of mRNA extraction, stabilisation, and amplification methodologies [36–40] have enabled previously intractable scenarios to be investigated, generating rich datasets describing host and pathogen responses to infection.

The first studies measuring transcriptional changes in host cells contrasted the gene expression patterns of macrophages after infection with different pathogens. For example, by comparing the macrophage responses to *M. tuberculosis* with six Gram-positive or Gram-negative bacteria, Nau et al. [41] defined a common macrophage activation signature and observed that interleukin (IL)-12 and IL-15 were not induced by *M. tuberculosis* infection. This distinguished the macrophage response to *M. tuberculosis* from other bacterial pathogens and suggested that *M. tuberculosis* may actively suppress macrophage pro-inflammatory processes. A similar approach has been employed to understand how events diverge between phagocytes and virulent or attenuated *M. tuberculosis* laboratory strains (H37Rv or H37Ra, respectively). Spira et al. [42] recognised a pro-apoptotic signature in alveolar macrophages after infection with H37Ra versus H37Rv, which was abrogated upon neutralisation of tumour necrosis factor (TNF). Thus, contributing to the hypothesis that virulent *M. tuberculosis* bacilli prevent macrophage programmed cell death mediated by TNF. In the converse experimental approach, Chaussabel et al. [43] contrasted the responses of different immune cell subtypes (monocyte-derived macrophages and dendritic cells (DCs)) to infection with the same pathogens. Such analyses have identified microbe-specific and cell-specific activation programmes that reflect the multi-factorial interplay of immune cell colonisation, providing insight into novel pathways influencing bacterial control and evasion of these processes by pathogens. We used the disparate ability of human monocyte-derived macrophages and DCs to control *M. tuberculosis* infection to compare the transcriptional responses of both host cell and infecting bacilli to the development of permissive and non-permissive intracellular microenvironments (in macrophages and DCs, respectively) [44]. This study revealed that a number of zinc-responsive genes were up-regulated in macrophages after *M. tuberculosis* infection and that correspondingly *M. tuberculosis* genes encoding heavy metal transporters were also induced after phagocytosis. Extension of this work demonstrated that zinc accumulation in phagosomes was toxic to engulfed non-tuberculous bacteria, uncovering a new macrophage anti-microbial strategy, and that *M. tuberculosis* bacilli are able to avoid zinc poisoning by inducing metal cation efflux pumps during macrophage infection [45].

Exploring macrophage transcriptional adaptations to *M. tuberculosis* infection may also contribute to understanding how genetic background influences susceptibility to tuberculosis. Keller et al. [46] compared the responses of murine bone marrow-derived macrophages extracted from C57BL/6 and

BALB/c (representing *M. tuberculosis*-resistant) with DBA/2 and CBA/J (*M. tuberculosis*-susceptible) mouse strains. The authors highlighted over 100 genes whose expression during the early phases of infection may, in part, be responsible for the contrary progression of tuberculosis disease in these genetically distinct mice lineages. Thuong et al. [47] extended this concept to investigate human genetic susceptibility to tuberculosis, examining the transcriptional responses of monocyte-derived macrophages from patients with latent versus pulmonary tuberculosis to *M. tuberculosis* antigen stimulation. By combining gene expression profiling with single nucleotide polymorphism mapping, the authors showed that the function of chemokine (C–C motif) ligand 1, CCL1, may be associated with pulmonary tuberculosis in man. The combination of mRNA profiling and targeted gene inactivation is a powerful tool for recognising key host immune-mediators. Ehrt et al. [48] mapped the transcriptional signatures of bone marrow-derived murine macrophages from WT, iNOS-deficient, or phox91-deficient mice to *M. tuberculosis* infection, framing roles for nitric oxide synthase 2 (iNOS) and phagocyte oxidase (phox) in the control of *M. tuberculosis*. Furthermore, this strategy has been extended to characterise MyD88 (myeloid differentiation primary response gene 88)-dependent and MyD88-independent pathways of macrophage activation following *M. tuberculosis* infection and continues to delineate signal transduction pathways that mediate macrophage activity by comparing the signatures of *M. tuberculosis*-infected macrophages derived from panels of knockout mice [49]. In this way, unbiased gene expression analyses, providing a snapshot of changing host cell status, have enabled novel mechanisms affecting mycobacterial control to be elucidated.

On the other side of this hostile engagement, transcriptional profiling of *M. tuberculosis* during macrophage infection has revealed how mycobacterial metabolism adapts after phagocytosis and has acted as a bioprobe to survey the internal microenvironments that bacilli encounter (recently reviewed in [50, 51]). Schnappinger et al. [52] demonstrated that multiple gene families involved in the beta-oxidation of fatty acids were induced after murine macrophage infection, hypothesising that intracellular *M. tuberculosis* adopt a lipolytic lifestyle. This key feature of *M. tuberculosis* infection has been observed after infection of human macrophage-like THP-1 cells [53] and human monocyte-derived macrophages [44] and, together with the identification of a cluster of genes that likely metabolise cholesterol [54], highlights these metabolic changes as a common strategy for *M. tuberculosis* intracellular survival. In addition, the respiratory state of *M. tuberculosis* also changes during infection shifting from aerobic to microaerophilic to anaerobic respiration dependent on changes to the dynamic immune-environment [49]. The differential regulation of these metabolic and respiratory pathways together with stress-responsive regulons (such as *dosR* and *phoP*) is most clearly highlighted by comparing *M. tuberculosis* signatures from different intracellular environments. For example, the impact of interferon (IFN)γ-mediated murine macrophage activation [52] or the development of a non-permissive environment after DC infection [44, 55] results in similar *M. tuberculosis* adaptive responses. Rhode et al. [56] used concanamycin A to limit the acidification of murine macrophages to pH 7.0 versus pH 6.4, enabling the authors to differentiate acid-inducible *M. tuberculosis* genes. This study led to

the characterisation of an *M. tuberculosis* acid and phagosome-regulated locus (*aprA/B/C*) that is required for successful macrophage infection and whose expression is likely regulated by the PhoP/R two-component system [57]. Further evidence that the PhoP/R system is important for sensing and controlling *M. tuberculosis* responses to the intracellular environment comes from a study contrasting the transcriptional signatures of H37Rv with H37Ra (which contains a point mutation in *phoP*). Li et al. [58] observed that PhoP-regulated genes were differentially expressed between H37Rv and H37Ra during murine macrophage infection and concluded that the limited ability of *M. tuberculosis* H37Ra to react to the intracellular environment may account for its attenuated phenotype. Continuing this theme, the impact of genetic variation amongst *M. tuberculosis* clinical isolates on interactions with murine macrophages was explored further by Homolka et al. [59], who compared the intracellular gene expression profiles of 15 phylo-geographically distinct *M. tuberculosis* complex strains. The authors mapped genome-wide *M. tuberculosis* responses that reflected the diverse intracellular fates of these clinical strains and detailed a common programme of bacterial adaptation encompassing oxidative and/or nitrosative stresses and metabolic and physiological alterations. This analysis also detected clade-specific and strain-specific intracellular transcriptional patterns, providing a basis for further investigation into the consequences that geographical and genetic *M. tuberculosis* diversity may have on tuberculosis disease worldwide [59].

6 Transcriptional Profiling the Interplay Between Host and Pathogen

Global mRNA profiling of host and *M. tuberculosis* bacilli from invasive or non-invasive sampling of tissues offers an overview of the impact of mycobacterial infection as disease progresses. These studies explore the complex organ environments made up of diverse cell types and distinct populations of bacteria and survey the interactions between multiple cells. As such, these models are able to examine host–pathogen interplay in a heterogeneous environment capturing changes in cell populations as well as divergent gene regulation. Many of these studies are aimed at identifying biomarkers of tuberculosis disease states (reviewed recently by Walzl et al. [60]). For example, the mRNA abundance profiles of murine lungs and spleens after infection or vaccination have been used to follow changes in immune-mediators over time and to determine indicators of a protective response [61]. *M. tuberculosis* gene expression profiling from murine lungs has defined in vivo signatures and revealed divergent responses to infection contrasting immune-compromised with immune-competent murine hosts [62, 63]. Mehra et al. [64] described the temporal mRNA abundance profiles of NHP granulomas during early and late disease, observing that the expression of inflammatory markers significantly decreased in NHP granulomas through the course of disease. This approach has been translated to human tuberculosis disease by Kim et al. [65] who mapped the mRNA signature of human lung caseous granulomas using a combination of laser capture dissection

microscopy and microarray analysis. The authors distinguished a gene expression pattern reflective of a change in lipid metabolism in caseous granulomas that likely results in the accumulation of host lipids. Correspondingly, *M. tuberculosis* genes involved in fatty acid metabolism were induced in human lung sections (extracted during surgery for untreatable tuberculosis) compared to axenic culture [66]. Moreover, a transcriptional signature of enhanced cholesterol metabolism was observed in *M. tuberculosis* bacilli extracted from human sputum, where slow or non-replicating lipid body-positive "fat and lazy" bacilli were characterised [67]. The activation state of human immune cells at the site of tuberculosis infection has been sampled by harvesting cells from bronchoalveolar lavage fluid, providing a readout of immune cell migration and shifting immuno-regulatory processes during active disease [68, 69]. Systemic host responses to *M. tuberculosis* infection have been measured from whole blood to define factors that influence relapse of disease [70] or active versus latent infection [71]. Thus, whole genome approaches to understanding mycobacterial disease continue to generate novel hypotheses, recently illustrated by the unexpected discovery of a neutrophil-mediated type I-interferon signature in the peripheral blood of patients with active tuberculosis [72].

Transcriptional profiling the crosstalk between host immune cells and *M. tuberculosis* bacilli in vitro and in vivo has identified common and specific responses to phagocyte or *M. tuberculosis* genotype, revealing novel mechanisms of bacterial control and immune-modulation and providing an interpretive framework for future studies. The techniques to generate genome-wide datasets at DNA, mRNA, protein, and whole cell levels are now established; the challenge, and the focus of the remainder of this chapter, is to integrate these layers of information to build predictive models describing host–pathogen interactions. For example, a greater understanding of the order of events during infection, mapping how interactions change over time, combined with targeted gene knockout/knockdown approaches promises to further unravel this destructive host–*M. tuberculosis* relationship. Such approaches may expose the functional significance of genes whose roles are currently unknown and which make up around 40 % of *M. tuberculosis* genes differentially regulated intracellularly [59]. To do this effectively we need mathematical models that are capable of mapping and forecasting these dynamic interactions between host immune cells and infecting pathogen.

7 Systems Biology and Modelling the Dialogue Between Host and Pathogen

The modelling of host–pathogen interactions is being actively pursued [73]; however, this approach is still in its infancy. Although mathematical models have a long history in biological science [74], their widespread application is a more recent phenomenon, linked to the field of systems biology, that has emerged over the last 15 years. Modelling can be performed on many scales (from molecular dynamics to whole organisms) and the entities that are modelled can be discrete (number of

molecules) or continuous (concentrations, probabilities). Similarly, time (discrete time points, continuous time) and space (well-stirred solution, continuous concentration gradients, discrete neighbourhoods/microenvironments) can be considered in various ways. The choice of modelling method depends on the available knowledge and the phenomena that are to be investigated. Generally speaking, when the processes involved are known in sufficient detail, differential equations are often applied as they have been used extensively in the natural sciences, in particular physics, and are amenable to the mathematical analysis of large datasets. In a typical scenario, when the available knowledge is incomplete, discrete (variables and time) models are a good starting point. Modelling strategies used in host–pathogen systems biology have been reviewed by Forst [75], as has the use of models to complement experimentation by Kirschner and Lindermann [76]. The application of systems biology to tuberculosis research was reviewed by Young et al. [77]. Modelling host–pathogen interactions represents a particular challenge due to the multitude of different cell types that participate in the immune response to infection. Even if only direct connections between pathogens and host cells are considered the situation remains complex as infection can proceed in various ways. Since any modelling effort seeks to start with simple models, construction of models describing complex host–pathogen interactions has only begun in recent years.

8 Interaction Databases and Network Maps

Many models operate at the molecular level; therefore, it is a necessary first step to generate an overview of the possible interactions in the system. These may be taken from the relevant literature as well as interaction and pathway databases. A number of such databases exist and are detailed at http://www.pathguide.org [78]. Of particular interest is InnateDB [79], a database of interactions relevant to innate immunity in human and murine cells. Besides integrating data from external sources, InnateDB employs a curation team that uses the literature to specifically collect experimentally validated interactions in innate immunity. These interactions may be viewed in a pathway context mapping gene expression data onto them. This makes it possible to find pathways in which modulated genes are overrepresented. For this analysis, the pathways can be considered as models, because they represent the context in which interactions are thought to have a functional relevance. As an additional feature, InnateDB can use gene expression data to look for enrichment of transcription factor binding sites in up- and down-regulated genes. The putative TF-binding sites are mined from the cisRED database [80] which specialises in the prediction of these sites.

In addition to information about these molecular interactions, every modelling effort also requires data for model evaluation. This can be found in the literature or deposited in databases. Databases with particular relevance to host–pathogen interplay include http://www.macrophages.com, http://www.signaling-gateway.org [81], http://www.tbdb.org [82], or BugsBase (http://www.bugs.sgul.ac.uk/bugsbase).

These sites provide many types of datasets, in particular microarray, protein expression and protein regulation studies. Simple models built from interactions without specifying type or function may be generated and interrogated. For instance, Brodsky and Medzhitov [83] investigated targets of bacterial pathogens in protein–protein interaction networks of immune signalling. Their analysis suggests that pathogens which cause acute infection tend to target highly interconnected nodes of the network, while in chronic infections nodes with only a few connections are primarily targeted. Dyer et al. [84] surveyed the landscape of human proteins that interact with pathogens. Interestingly, the vast majority of interactions that they observed were from viral systems. They found that pathogens often target proteins that act as hubs, directly participating in a large number of interactions or involved in many different signalling pathways. At the next level of complexity, simple interaction networks may be annotated more richly to distinguish between the many different types of processes and components involved. This can be achieved in a standardised manner using existing ontologies and description standards (for example, gene ontology [85] and Systems Biology Graphical Notation [86]). In recent years, several descriptive models (pathway maps) relevant for host–pathogen interaction have been published [87–90]. These maps can be viewed as a kind of systematic knowledge representation which is complementary to classical review articles. In addition, it is often possible to overlay genome-wide data onto these maps for visualisation and analysis purposes. This provides a quick overview of the key features of the dataset and allows users to recognise interactions that may potentially form functional units in the experimental conditions tested. Although network maps cannot at present be used to calculate signalling outcomes or to make predictions about interference with the network, they serve as an excellent basis for new computational modelling efforts.

9 Models of Host–Pathogen Interactions

A basic model to predict cell-mediated immune-regulatory mechanisms during *M. tuberculosis* infection was proposed by Wigginton and Kirschner [91]. Ordinary differential equations were used to model the interplay between macrophages (resting *vs.* activated), *M. tuberculosis* (extra- and intracellular) and $Th_{0/1/2}$-cells as mediated by four cytokines (IL-4, IL-10, IL-12, and IFNγ). Most parameters were derived from published experimental data and if that was not possible their order of magnitude was estimated by sensitivity analysis. The main goal of this study was to explore which elements of the host–pathogen dialogue led to active disease or latency (and possible reactivation). Extensive model analysis concluded that if the initial immune response was dominated by Th_2-type cells, then the infection resulted in active tuberculosis. The prediction was not definitive when the initial immune response was predominantly mediated by Th_1-type cells. This model was extended by Sud et al. [92] to investigate the effects of CD8+ T-cells on disease outcome. The authors found that the cytotoxic and IFNγ-producing subpopulations of CD8+

cells contribute differently to the outcome of disease and that disease may still be controlled if either subpopulation is removed. However, if all CD8+ T-cells are deleted then the result was always active disease. As a further extension of these two models, Marino et al. [93] investigated the reactivation of tuberculosis following anti-TNF treatment and suggested several strategies for minimising the reactivation risk during anti-TNF treatment. In a closely related model, partly constructed from those previously mentioned, Day et al. [94] explored the effect of early appearance of classically activated macrophages in the lung upon *M. tuberculosis* infection. Under normal conditions, alveolar macrophages were considered to be alternatively activated and hence have reduced pro-inflammatory potential. The simulations showed that a reduced time delay for classical activation led to lower bacterial loads; this model was used to investigate the effectiveness of IFNγ therapy aimed at reducing this delay.

Raman et al. [95] developed a qualitative model of host–pathogen crosstalk in tuberculosis geared towards the prediction of disease outcome which can either be active disease, persistent infection or bacterial clearance. The interactions, between *M. tuberculosis* and different types of immune cells (innate and adaptive), were primarily mediated by cytokines and *M. tuberculosis* virulence factors; however, the molecular basis of these effects was included only in limited detail. Most interactions were modelled as Boolean functions, but there were additional parameters of time (*e.g.* onset of adaptive immunity) and the growth/clearance rates for *M. tuberculosis* affecting bacterial load which were modelled as continuous variables. For model simulation, an asynchronous update rule was used with each time interval corresponding to roughly 1 day. The primary result, the statistical evaluation of disease outcome, was determined after multiple (*e.g.* 100) model runs. This scheme made it possible to systematically study how changes in parameters or node deletions modified disease outcome. For instance, disabling phagocytosis always resulted in active disease which would only occur in 13 % of simulation runs with default parameters. Although the latter result was expected, the model may also be utilised to build more intricate predictions. For example, the knockout of TGFβ or IL-10 increases bacterial clearance, although these cytokines are typically classified as anti-inflammatory. This highlights that such simple classifications may not always be helpful because the effects of many signalling molecules are strongly dependent on the context. Similar to the previous study, Thakar et al. [96, 97] have developed models for infection of the lung by two *Bordetella* species. In the first version of the model [96], the authors concentrated on discrete dynamics to investigate basic effects like persistence and clearance of the bacteria. As the approach used by Thakar et al. [96] is analogous to that used by Raman et al. [95] described above, we concentrate here on the second version of this model published in 2009 [97], which uses a hybrid dynamic approach to better describe available quantitative data. In the hybrid dynamic model, each node is described using both a discrete (Boolean) and a continuous variable. The value of a discrete variable depends on whether the continuous variable exceeds a certain threshold, with the threshold being a parameter of the model. To describe the time evolution of the continuous variables, the Boolean rules from the first model are used for the activation of the nodes. The deactivation

is modelled with separate linear decay terms which together yields a system of piecewise linear differential equations. In this hybrid model the parameters do not directly correspond to kinetic or binding parameters that are usually considered in differential equation models. In order to identify actual parameter values, a large range was sampled and only such parameter combinations were selected that reproduced certain well-known qualitative features of the infection dynamics. The parameters found in this manner were analysed further, searching for correlations to develop novel hypotheses for future experimental testing.

While the previous models consider the interactions of pathogens with different immune cell populations in an abstract manner, the model developed by Franke et al. [98] describes the crosstalk between *H. pylori* and epithelial cells in molecular detail. *H. pylori* is able, in a CagA-mediated process, to translocate into the host cell, triggering several events. In particular, the receptor tyrosine kinase c-Met, which normally plays a role in the context of human growth factor (HGF) signalling, may be recruited by CagA. The main target of CagA-induced immune-modulation is considered to be the MAP kinase ERK1/2, which is activated by stimulation with HGF or CagA. The interactions in this model are represented by Boolean functions and as a first step the interaction graph underlying the logical network was analysed. In this graph, only the information concerning positive and negative regulatory events was retained. The dependency matrix, which collects network-wide interdependencies, was calculated on the basis of the interaction graph. This revealed that HGF can exert both activating and inhibiting influences on ERK1/2, while CagA acts solely as an activator. Following on from this, the logical states in the network after stimulation with either HGF or CagA were determined, which showed that the signal was propagated through partially distinct pathways. This resulted in the systematic search for interventions that would prevent ERK1/2 activation upon CagA stimulation without affecting HGF signalling. Several of the predictions generated in this manner were then tested and confirmed. This indicates that the model captured important features of a real signalling network and could thus be used to generate new hypotheses for experimental testing. Additional Boolean models of within-host immune interactions are reviewed by Thakar and Albert [99]. To summarise, modelling complex host–pathogen interactions is well under way; however, one particular challenge remains the detailed modelling of the gene expression layer. Although many models contain transcription factors and interactions with their binding sites, these are currently far from comprehensive for both host and pathogen.

10 Future Perspective

The crosstalk between *M. tuberculosis* and its human host is both complex and dynamic, as such genome-wide approaches are invaluable tools for the unbiased discovery of novel interactions which serve to inspire testable hypotheses. Computational models are becoming increasingly useful for mapping and interrogating these multi-layered datasets, as evidenced by the chapters in this book. Advances in single cell manipulations

together with the development of relevant infection models will enable single cell interactions between host and pathogen to be characterised, revealing the population dynamics of *M. tuberculosis* infection. Such analyses will aid the development of new drugs and vaccines which are desperately needed to reduce the burden of tuberculosis disease worldwide. Recent exciting progress classifying disease states and exploring the impact of genetic variation in both *M. tuberculosis* and human populations strengthens the prospect of elucidating valuable biomarkers of disease and determining the genetic basis of disease susceptibility. Finally, the emerging significance of small regulatory RNAs and epigenetics in the field of infectious disease promises to uncover novel mechanisms affecting immune-modulation, offering multiple opportunities for future intervention.

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