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Computational modelling of mycobacterium infection and innate immune response in zebrafish

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3 Multi-scale Petri net model of the bacterial–macrophage interaction

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O, Arbeid	Oh, Labor
Niet de partijen	Not the parties
Niet de stellingen	Not the propositions
Niet de woorden	Not the words
Niet het zijn	Not the being
Leven of sterven	To live or die
Winnen of verliezen	To win or lose
Het is alles een	It is all one
Recht of waarheid	Law or truth
Blijft alles het zelfde	Everything remains the same
Zonder arbeid is er geen	Without labor, there is none
Arbeid alleen kost al dit leven	Just labor costs all this life
Leven is dus arbeid alleen.	So life is just labor.
Marinus van der Lubbe, Nederland (1909 – 1934)	Marinus van der Lubbe, Netherlands (1909 – 1934)

Abstract

A primordial step in the modeling process is refinement. This becomes feasible once more information about the phenomenon under study, i.e. bacterial infection, is collected. Therefore, we can extend the model allowing more complexity according to the information acquired. The previous chapter has indicated the direction for the refining process, where increasing the complexity is directed to modeling the interactions that occur at cellular and molecular level. In this chapter we, therefore, gather the information about the complex pathways related to the infection persistence modeling them in a hierarchical structure. The result is a model that can perform qualitative simulations in order to mimic the alternatives that might occur during the infection.

3.1 Introduction

Tuberculosis (TB) is the second greatest killer disease worldwide due to a single infectious agent: *mycobacterium tuberculosis* (*Mtb*) [129]. Effective vaccination against tuberculosis is a challenge; a better understanding of the host-pathogen relationship provides an important key for new treatments. The host innate immune response is the first line of defense against invading microbes. It recognizes the pathogen in the first stage of infection and initiates an appropriate immune response. Therefore it has been the subject of much scientific research involving mycobacterial infection [24, 30, 69, 71, 110, 111].

The complex interactions between bacteria and the immune cell involve various structures and processes that control, activate and inhibit proteins and signaling pathways in a dynamical system that determines the outcome of an infection [20]. A systematic approach to modeling these interactions should help to comprehend the events that occur between the host and pathogen [124]. Different methods have been used to model the mycobacterial infection process: Gammack *et al.* [41] provided a mathematical model based on Ordinary Differential Equation (ODE) to investigate the early and initial immune response to *Mtb*. Such work has inspired Segovia-Juarez *et al.* [108] to implement the ODEs that regulate the interaction between host and pathogen using an agent-based approach, and Warrender *et al.* [124] use the CyCells simulator tool to simulate the interactions in Early *mycobacterium* infection.

Mathematical models, like those based on differential equations, are difficult to obtain and analyze when the number of interdependent variables grows and when the relationship depends on qualitative events. The computational models used for this problem offer an additional avenue for exploring the infection dynamics through the visualization of a specific behavior simulation. However, in both cases the interactions between bacteria and the immune cells and their structures are not intuitively described. The interactions are embedded in programming code and/or described in rules, which are not straightforward to interact with, nor comprehend their relationship.

A graphical representation of the interactions and influences among the various molecular and cellular components that involve the bacteria and host immune cells that also captures the dynamics of the system should be very useful. The framework of Petri nets represents a well-established technique in computer science for modeling distributed systems [105] and they have been successfully used to model biological behavior. Heiner *et al.* [52] propose a methodology of incremental modeling using Petri nets. They develop and analyze a qualitative model of the apoptotic pathway. In our previous work [19] we have developed a qualitative model of the mycobacterial infection process and the innate immune response. We modeled the cell dynamics level, characterized by the steps that are involved in the *Mycobacterium marinum* infection and granuloma formation in zebrafish.

In this paper, we extend our model and focus on interactions between the bacteria and the host immune cells - specifically the macrophages - in a multi-scale model. We identify and connect the important pathways involved in the host-pathogenic interactions that act over different scales (molecular, intracellular, and intercellular) during the innate immune response. The model captures the quintessential functional processes of the macrophage upon exposure to mycobacteria, their interconnections, subsequent signals and activation of the immune response. It provides a visualization of the signaling pathways that the host immune cell utilizes to terminate the infection as well as the way the pathogen exploits the pathways of the macrophages to enhance its intracellular survival persistence. This Petri net model makes it possible to perform “what-if” situations as part of the experimentation, simulating possible pathway disruptions and the consequences to the infection process. In this paper, we demonstrate the power of the Petri net formalism in modeling signaling and metabolic pathways that are involved in the host-pathogen interaction in a multi-scale model. We simulate and animate three different dynamics to mimic the alternatives that might occur once a bacterium is phagocytized by a macrophage and persists in infection. As a next step we plan to consider a qualitative validation of the model so as to confirm consistency and correctness of its biological interpretation.

3.2 Mycobacteria interaction with macrophages

Macrophages play rather contradictory roles in infection and disease as they are likely the first host immune cells to respond to invading mycobacteria, and yet aid in subsequent dissemination of the bacteria [37]. The successful parasitization of macrophages by mycobacteria involves the inhibition of several host-cell processes, which allows the bacteria to survive inside the host cells. The host processes that are inhibited by the pathogenic bacteria include fusion of *Phagosome* with *Lysosomes*, antigen presentation, apoptosis and the stimulation of bactericidal response [68].

Mycobacterial cells release a mixture of lipids and glycolipids that interfere on the macrophage response towards elimination and enabling bacterial survival [104]. Mannosylated Lipoarabinomannan (ManLAM) is one of the major modulators of phagosome maturation [112]. It prevents fusion of mycobacterial phagosome with the late endosome and lysosome by inhibiting the Calmodulin- Ca^{2+} phosphatidyl-inositol-3 kinase [40]. Ca^{2+} also has an influence on the apoptotic pathways since it increases the permeability of mitochondrial membranes releasing pro-apoptotic elements to facilitate apoptosis [68]. ManLAM also influences the apoptosis by phosphorylating the apoptotic protein Bad leaving the anti-apoptotic protein Bcl-2 free which inhibits caspase activity and functions as an anti-apoptotic regulator [1].

Macrophages and T cells produce many cytokines that promote or inhibit protective response to the mycobacterial infection. An important family of cytokines is the interleukin-10 (IL-10), which

regulates the pro-inflammatory (PICs) and anti-inflammatory (AICs) cytokines. The bacteria can limit macrophage apoptosis by inducing the production of IL-10 which blocks the synthesis of Tumor-Necrosis Factor (TNF), a stimulator of apoptosis in infected macrophage [84, 107]. It is likely that bacteria prevent apoptosis in the early phase of infection to allow them to replicate efficiently. However, in the later phase, they induce or are unable to prevent cell death, which might facilitate their systemic dissemination through uptake into immune cells [68].

3.3 Cell-cell host pathogen interaction

The modulation of host signaling mechanism is a dynamic process requiring mycobacterial components that trigger or inhibit the host response such as the fusion of Phagosome with Lysosomes, antigen presentation, apoptosis and stimulation of bactericidal responses due to the activation of pathways that leads to the bacterial survival. The immune cells can identify the pathogen through Pattern Recognition Receptors (PRRs), which are found on the cell surface, on the endosomes and on cytoplasm. It triggers a cascade of events that leads to proinflammatory and antimicrobial response through the phagosome maturation pathway. Van der Vaart et al. reviewed the PRRs that identify invading microbes, as well as the innate immune effector mechanisms that they activate in zebrafish embryos [120]. The maturation of the phagosome forms the late-phagosome, which fuses with the lysosome forming the phagolysosome. It can digest the pathogen and leads to the bacterial death [11, 12, 102]. The mycobacteria are using several strategies to avoid the maturation of the phagosome and the key contributor is mannosylated lipoarabinomannan (ManLAM), a glycolipid of the mycobacteria cell wall. ManLAM is involved in the inhibition of phagosome maturation by inhibiting both calcium (Ca^{2+}) concentration of increase in the macrophage as well as the concentration of Calmodulin- Ca^{2+} phosphatidylinositol-3 kinase (PI3K) which is responsible for leading the maturation of the phagosome and driving the fusion with the lysosome [40, 76, 122]. To accomplish complete arrest and prevent the phagosome maturation, a second mycobacterial macromolecule, SapM, is released degrading the existing Phosphatidylinositol 3-phosphate (PI3P), a phospholipid found in the cell membrane involved in the phagosomal maturation [97]. A schematic representation of the phagosomal maturation arrest by the pathogenic mycobacteria is given in **Figure 3.1**.

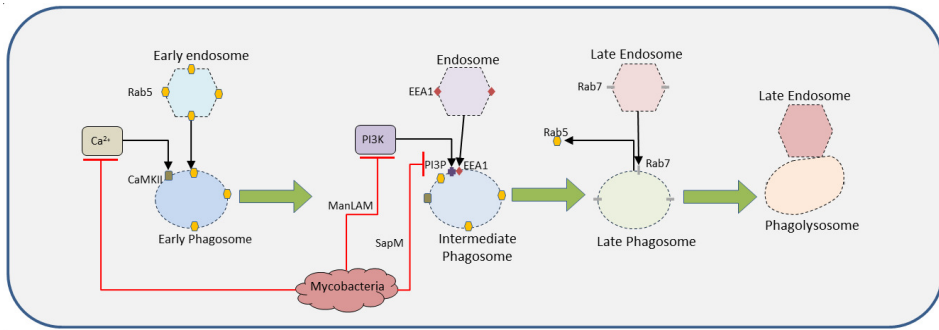


Figure 3.1: Schematic overview of the phagosomal maturation pathway blocked by pathogenic mycobacteria according to Koul et al. [68]. Nascent phagosome acquires Rab5 recruiting PI3K that generate PI3P. Pathogenic mycobacteria block the rise in cellular Ca²⁺, recruitment of PI3K to the phagosome and degrading PI3P through Sap-M

When the immune cell is not able to kill the bacteria through the phagolysosome, the macrophage activates the apoptosis thereby programming its own death and signaling to other defense mechanisms. Once the maturation fails, the apoptotic programme is mainly activated by the extrinsic apoptosis pathway, which is initiated by the binding of ligands to death receptors. The apoptotic program also activates the intrinsic pathway, which involves translocation of cytochrome-C from mitochondria to the cytosol. The activation of the caspase cascade and degradation of genomic DNA are characteristics of apoptotic cell death [68]. Mycobacteria alter host apoptotic pathways interfering on the intrinsic death pathway preventing the increase in cytosolic Ca²⁺ concentration. Mycobacteria also inhibit caspase activity and functions by stimulating the phosphorylation of the apoptotic protein Bad [76, 103]. Mycobacteria limit macrophage apoptosis by inducing the production of cytokines such as interleukin-10 (IL-10). These cytokines interfere with one of the apoptosis stimulators of the macrophage in the extrinsic apoptosis pathway, the tumor-necrosis factor- α (TNF- α) [25, 84]. Mycobacteria take advantage of blocking these defense mechanisms of macrophages - phagocytosis and apoptosis - to proliferate inside the cell till a necrosis breakdown. Therefore, bacteria disseminate the infection through the other immune cells that aggregate with that particular infected macrophage to take over the infection. The apoptotic pathway is depicted in **Figure 3.2**.

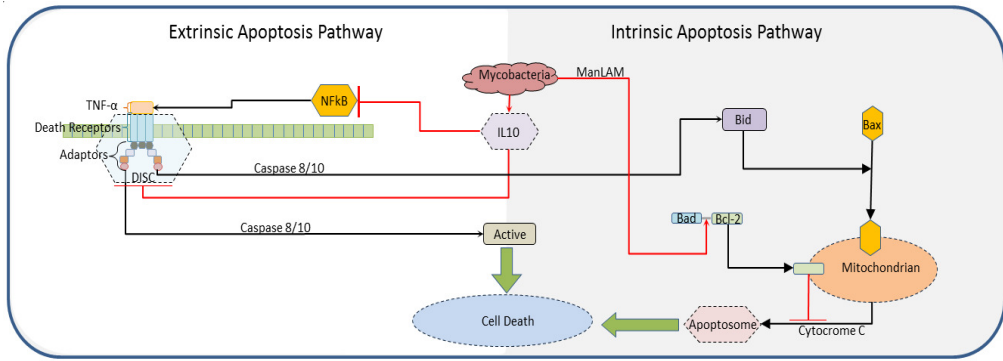


Figure 3.2: Apoptotic pathway inhibition by pathogenic mycobacteria according to Koul et al. [68]. Pathogen mycobacteria interfere in intrinsic apoptotic pathway suppressing Ca^{2+} and releasing Bcl-2. In the extrinsic apoptotic pathway it inhibits binding ligands and DISC formation.

3.4 Molecular host pathogen interaction

At the molecular level the most important interactions occur in both the phagosomal maturation pathway and in the apoptosis pathway. In both cases, the mycobacteria interfere in different ways to guarantee their survival and proliferation. Ca^{2+} is a key messenger that is released from intracellular storage. An increase in cytosolic Ca^{2+} concentration promotes the phagosomal maturation process by regulating calmodulin and the multifunctional serine/threonine protein kinase CaMKII [76]. CaMKII is important to PI3K activation and recruitment of early endosomal antigen 1 (EEA1) to the phagosomal membrane, which is extremely important in the process of phagosomal maturation. PI3K is also essential for the production of the lipid regulator phosphatidylinositol 3-phosphate (PI3P), which forms a ligand together with EEA1 leading to an intermediate phagosome. The intermediate phagosome matures to the late endosome after EEA1 dissociation and acidic expression due to accumulation of the proton-ATPase [39, 114]. Through releasing ManLAM, the mycobacteria inhibit the rise of the Ca^{2+} concentration in macrophages and also the PI3K activation. It prevents the generation of PI3P degrading the existing PI3P by the action of SapM.

Despite the fact that phagosome fail to fuse with the lysosomes to degrade the bacteria, pathogen-derived material is released in the host cell lysosomes and on cell surface of the infected macrophage, which can induce the apoptosis process [106]. Mycobacteria influence the host apoptosis through several mechanisms, which interfere in the intrinsic and extrinsic apoptosis pathways. The cytosolic Ca^{2+} facilitates apoptosis by increasing the permeability of mitochondrial membranes. This process promotes the release of pro-apoptotic elements such as cytochrome-C. In the cytosol, cytochrome-C associates with procaspase-9 and apoptosis protease forming a signaling complex called the apoptosome, which activates the induction of apoptosis [70]. ManLAM interferes in the intrinsic apoptosis pathway not only by inhibiting the concentration of Ca^{2+} but also

through stimulation of the phosphorylation of the apoptotic protein Bad. The Bad phosphorylation leaves BCL-2 free by which the release of cytochrome c is prevented.

The extrinsic apoptosis pathway is induced by Toll-like receptors (TLRs) that identify the virulence mycobacterial pathogen. Those receptors trigger the synthesis of tumor-necrosis factor- α (TNF- α) - a stimulator of apoptosis – through the TLR signaling pathway. To do so, an important adaptor factor protein, the Myeloid differentiation factor 88 (MYD88) recruits a family of kinases (IRAK) that will form “Myddosome” signaling complex [119]. This signaling complex activates nuclear factor κ B (NF- κ B) to transcribe target gene to synthesize TNF- α [68]. The tumor necrosis factor binds with death receptors leading to a cascade of events that will release caspase 8 and 10. It also leads the formation of a death-inducing signal complex (DISC) resulting on the apoptotic vesicles [70, 132]. Pathogen mycobacteria interfere in this process by inducing the production of immunosuppressive cytokine interleukin-10 (IL-10). IL10 inhibits the phosphorylation of NF- κ B, and thereby the synthesis of TNF- α . It also inhibits the DISC formation and consequently, the extrinsic apoptotic pathway fails.

3.5 Petri net model of the bacterium–macrophage interaction

We have formulated a Petri net model of the processes triggered in the macrophage in response to mycobacterial infection. The model is based on an extensive literature survey (cf. Sections 3.2 to 3.4) and on extending our previous model [19]. The model captures the interactions between the immune cell and the pathogen once a bacterium is phagocytized. The model is hierarchical and has three different levels of representations to mimic the signal processing that activates/inhibits the pathways related to the macrophage response to the bacteria. The first level models the overall actions from the system started after the phagocytosis and it represents the cell-cell interaction between the macrophage and the bacteria. The second level representing the intracellular interaction models two important signaling pathways: the Phagosome Maturation which is responsible for the degradation of the infection through antimicrobial components; and the Apoptotic Pathway which is the macrophage mechanism responsible to resolve the infection in response to virulence factors. It represents an alternative way to the phagolysosome. The third level represents the molecule-molecule interactions that occur on the level of Phagosome Maturation and Apoptotic pathways.

To model the host-pathogen interaction we use a qualitative Petri net as implemented in the Snoopy software [50] using maximal concurrency semantics. All formal definitions can be found in **Appendix A**. The pathways described in Section 3.2 to 3.4 represent a complex process involving various host-bacterial factors in a heavy cross-talk interaction. To get a consistent view of the entire interaction process, we express the most important reactions simplifying the pathways at different levels of abstraction. We define each biochemical compound or receptor as a place. The relations

between biochemical substances are basically represented by transitions with corresponding arcs modeling biochemical reactions, inhibitions/degradations (using inhibitor arcs) or signaling/catalytic atomic events (using read arcs). To hierarchically connect the sub-nets we use coarse transitions and coarse places structuring all the levels as a tree as shown in **Figure 3.3**. The top level (the root) models the overall view of the system, starting by interactions that occur in the cellular boundary and its consequences. It is connected to the sub-nets (mid-level) through coarse transitions which link to the molecular level modeled in coarse places (the leaves of the tree). This structure increases the granularity of the model along the levels. Deeper is the level more detailed is the sub-net. **Table 3.1** describes each sub-model implemented hierarchically and the respectively level of interaction.

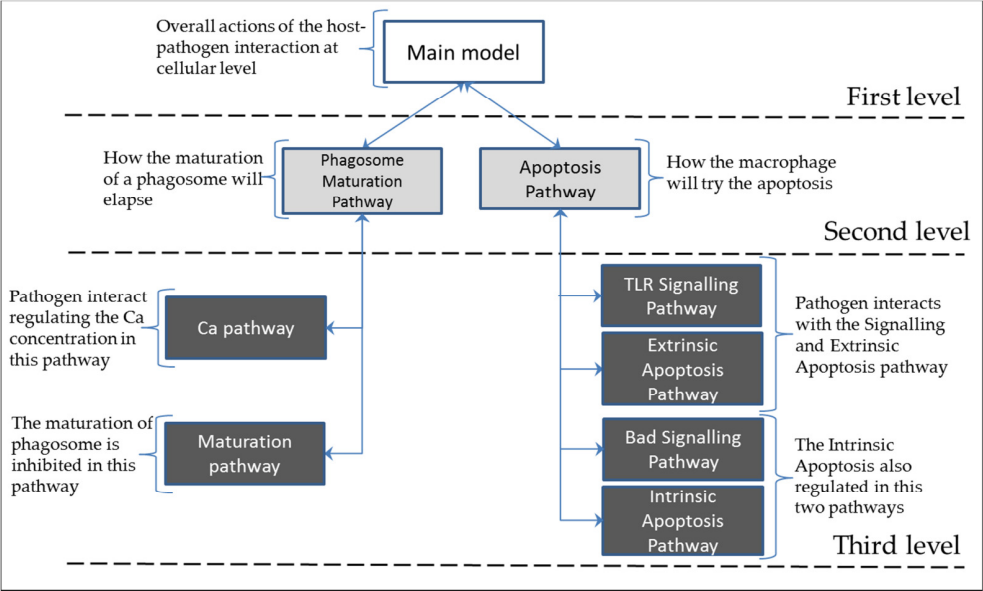


Figure 3.3: Hierarchical structure of the net. The three levels are implemented in independent interconnected sub-nets

Table 3.1: Set of places per hierarchical structure and the scale in which they occur

<i>Level</i>	<i>Scale</i>	<i>Description</i>
Main model	Intercellular	Models the states of the macrophage and the interaction that occur at the intercellular level.
Phagosome Maturation Pathway	Intracellular	Implemented by the coarse transition Phagosome_Maturation_Pathway , it models the interactions at the intracellular level that triggers the phagosome maturation process. It connects the molecular scale with the intracellular scale.
Apoptosis Pathway	Intracellular	Implemented by the coarse transition Apoptosis_Pathway , it models the interactions at the intracellular level that triggers the apoptosis process. It connects the molecular scale with the intercellular scale.
Ca_pathway	Molecular	Coarse place that models the molecular interactions that produce/inhibit calcium and CMKII.
Maturation_pathway	Molecular	Coarse place that model the molecular interactions that leads/inhibits the maturation of the phagosome process.
TLR_Signaling_Pathway	Molecular	Coarse transition that models the molecular interactions that leads to the activation/inhibition of pro-inflammatory cytokines.
Bad_Signaling_Pathway	Molecular	Coarse transition that models the molecular interactions that triggers/inhibits the phosphorylation of Bad apoptotic protein.
Extrinsic_Apoptosis_Pathway	Molecular	Coarse place that models the molecular interactions related to the extrinsic apoptosis process.
Intrinsic_Apoptosis_Pathway	Macrophage	Coarse place that models the molecular interactions related to the intrinsic apoptosis process.

3.6 Model definition

We start the modeling with the interaction between the bacteria inside the macrophage once it is in the host. The first level of our Petri net model is given in **Figure 3.4**. The input place **Infected_macrophage** represents this situation. The sequence of interaction events happens once there are bacteria infecting the macrophage. This process is represented in the net by a token that is present at the input place, detected by three reading arcs that trigger the interactions. The macrophage uses the PRRs to detect the presence of the pathogen and starts the phagosome maturation process. The bacteria start its protein secretion system and counter attack by releasing SapM to degrade existing PI3P in the cytosol and ManLAM to interfere in the maturation of the phagosome. The maturation of phagosome is modeled in a deeper level by the coarse transition **Phagosome_Maturation_Pathway**. ManLAM also interfere in the apoptosis process, which is modeled in a deeper level in the coarse transition **Apoptosis_Pathway**. The presence of ManLAM triggers the macrophage production of the cytokine IL10 and also interferes in both pathways. **Phagosome_Maturation_Pathway** interacts with **Apoptosis_Pathway** releasing calcium and bactericidal material that was not degraded by the maturation.

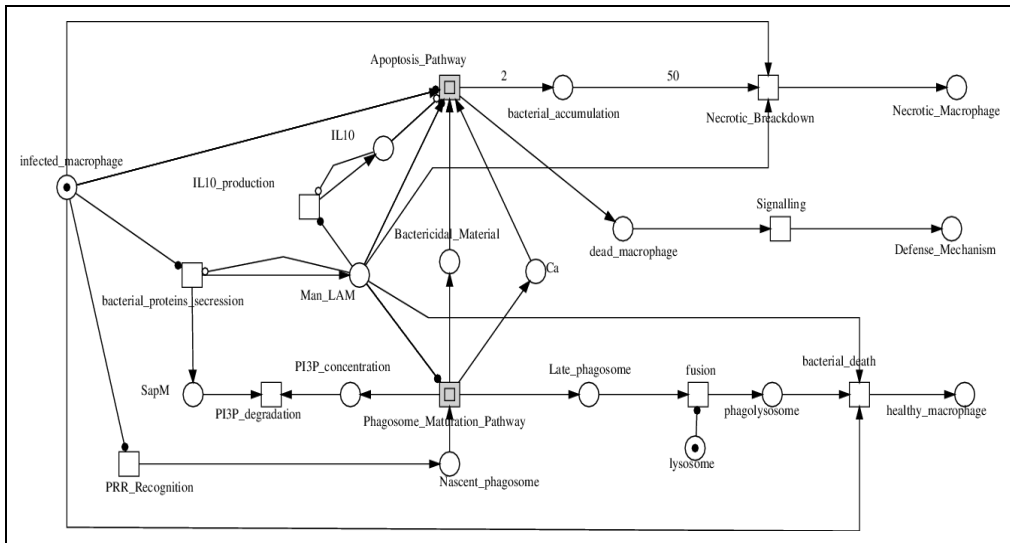


Figure 3.4: Petri net of host-pathogen interaction at the top (root) level. The coarse transitions: **Phagosome_Maturation_Pathway** and the **Apoptosis_Pathway** contain the second level of the model and are represented here by a double square.

In our model there are three different scenarios: The phagosome maturation occurs in the **Phagosome_Maturation_Pathway**, leading to a late phagosome that will fuse with lysosome digesting the bacteria, and turning the macrophage healthy. The second scenario can occur if the maturation fails. The apoptosis process in the **Apoptosis_Pathway** is triggered, leading to a dead macrophage that will signal for another defense mechanism. The third scenario occurs when both pathways (maturation and apoptosis) are failing at the molecular level. In that case the bacteria proliferate and accumulate in the macrophage till a necrosis breakdown, releasing all the pathogenic material to the surrounding cells. To represent the proliferation and accumulation of bacteria, we use weighted arcs that double the amount of bacteria, as accumulated in the place **Bacterial_accumulation**. The breakdown of the macrophage occurs when it reaches a threshold of 50 bacteria, thereupon a weighted arc fires the transition **Necrotic_breckdown**. Here we should note that the weighted arcs, with weights 2 and 50, are examples to express the idea of bacterial proliferation.

Following the hierarchical tree, at the second level we have: **Phagosome_Maturation_Pathway** and **Apoptosis_Pathway**. They are two sub-nets, which basically connect the cellular interaction (top level) with the molecular interactions at the biochemical pathways implemented in the coarse places (the branches of the tree). **Figure 3.5** depicts these sub-nets respectively. At this level we have the signaling started in the cell surface (first level) that will trigger the production/interaction between molecules. For example, the production/releasing of calcium is triggered by the PRRs and this process occurs at **Ca_pathway**; the PIP3 concentration and bactericidal material that are not degraded at the **maturation_pathway** and interact with the top level. We also have the interaction between the cytokine IL10 from the top level with the pro-inflammatory cytokines that will interfere in the TNF- α in the **Extrinsic_Apoptosis_Pathway** and ManLAM interfering in the BCL2 activation, which will act in the **Intrinsic_Apoptosis_Pathway**.

experience the events that can occur in the model. For inspection and perusal, the animations can be found at <http://bio-imaging.liacs.nl/galleries/eptn-infection/>.

To validate the model, it is necessary to define validation criteria for a consistency check. To do so, we have to consider that our model is based on a heuristic procedure of collecting information from the literature, perhaps with different interpretations, modeled from the process perspective (top level) down to the molecule perspective (leaves). We built a large model composed of sub-models and to provide a complete analysis, it is necessary to verify each component individually and then the system as a whole, which increases the complexity of the validation even with computational support. Basic qualitative behavior properties can be checked using the Charlie analyzer tool [126]. Heiner *et al.* have used as example, p-invariants and t-invariants to analyze case studies in biochemical pathways in [51]. We started to analyze the structural and behavior properties of our model based on results from the Charlie tool which should then be biological meaningful. As a first result, we found that our model is not structurally bounded and not reversible. This implies that indeed the net allows for the proliferation of the bacteria and the infection process is not reversible.

3.8 Conclusion

In mycobacterial infection, the dynamics of the interactions between the host and bacteria form a complex system involving numerous activations, inhibitory and control structures that determine the outcome of the infection. A systems approach is essential to comprehend the significance of the multiple events that occur simultaneously among the various molecular and cellular components of the host and pathogen.

Here, we seek to model the interaction of the macrophage upon exposure to pathogenic mycobacteria, capturing important functional processes and their interconnections including signaling and activation/inhibition of the immune responses on different levels of abstraction. The Petri net formalism has proved to be a useful modeling approach to describe and interconnect different abstract levels into a large and extensive model [52, 79] In Chapter 2 we have developed a Colored Petri net model to explore the early mycobacterial infection and the immune response, modeling the steps that regulate the infection process. In this chapter we focus on the processes occurring in different scales, from the cell and descend to molecular interactions relevant to the infection process. Therefore, we use a qualitative Petri net modeling different pathways in sub-nets, interconnecting them in a hierarchical structured model. The model provides a visualization of the processes occurring at multiple scales using levels that can be operated independently. It also describes the interconnections and signals that influence the host pathogen interaction.

In the model we express, at different levels of abstraction, the details that are involved in the macrophage-mycobacterium interaction. Information about the proteins released by the bacteria,

their interference in the immune response and the pathways involved in this process are observed in our model. It is possible to visualize the dynamics of the molecular and cellular interaction as well as analyze different scenarios performing “what-if” simulations as part of the experimentation. In this manner, different animation/simulation can be accomplished. The model represents the information about host-pathogen interaction available in the literature but the scalability of our model allows extension to a more complete system.

As part of the modeling process, we used the Charlie analyzer [126] to check basic properties of the model and its consistency. As a next step, an extensive analysis of more structural and behavior properties is necessary to validate the model. We also intend to extend to a quantitative model where, with support of experimental data rather than the examples we used until now. In this manner we would be able to perform quantitative simulations and behavior analysis. This can contribute, for example, in the prediction of results from new experiments and generation of further hypotheses about the innate immune system response to mycobacterial infection. Another challenge is to combine the models implemented in different classes of Petri nets in one system. One solution is to adapt each model in a Hybrid Petri net, or abstract the models in a Nets-within-Nets approach where the communication of the tokens occurs via predefined interfaces which are dynamically bounded [57].

In summary, in this chapter we have presented a model that explores the interaction between mycobacterial pathogen and macrophage, modeling the dynamics in three different level of abstraction while interconnecting them in a hierarchical structure. We have checked the structural behavior of our model through an analysis tool. The interplay of hierarchical levels and qualitative/quantitative information has the potential to develop a powerful tool for the research in tuberculosis disease.