WP1 - Modelling phagocytosis

Deliverable 1.1 – Initial Model

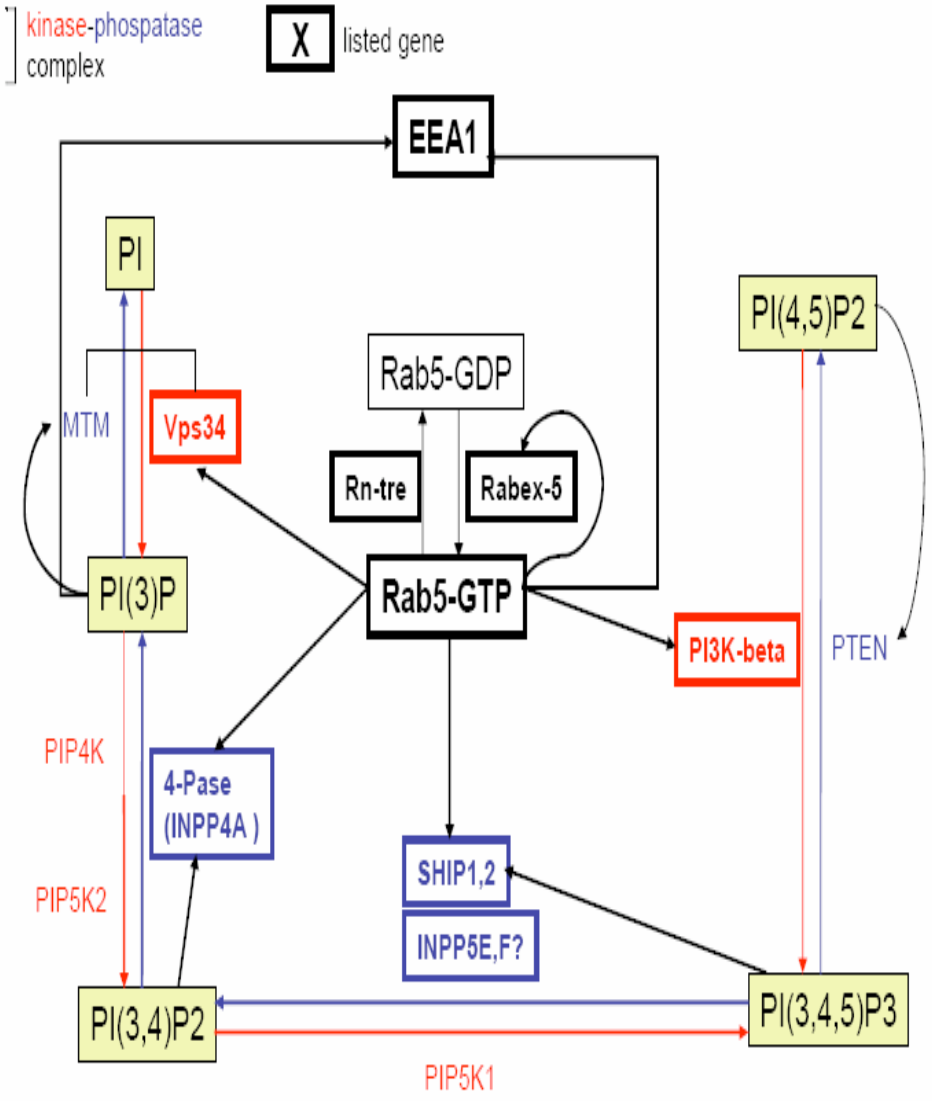
WP1 has experienced some delays in the progress of the project due to a number of factors. The long term illness and subsequent tragic death of the mathematician Professor Jaroslav Stark, coupled with the late start of the research fellow at ICSTM (April 2009) and delays in identifying suitable data for the initial modelling has meant that progress has been slow. However, a biomathematician, Dr. Vahid Shaherezei, has now been recruited to the project and has helped to take the modelling forward.

The initial model, developed at ICSTM, focuses on phosphoinositides (PIPs) relevant in phagocytosis, especially during the early Rab5 stage. PI(3)P, phosphatidylinositol 3-phosphate production is regulated by Rab5 through a dual mechanism – by directly phosphorylating PI via Vps34 and by an enzymatic cascade of PI3K-beta, PI 5-, and PI 4-phosphatases (Shin et al., 2005). This interesting dual production, lead us to focus on a small, but important component of this network – the kinase-phosphatase complex (Vps34-MTM). Several similar kinase-phospatase complexes have been identified so far, although some of them are hypothetical (see figures).

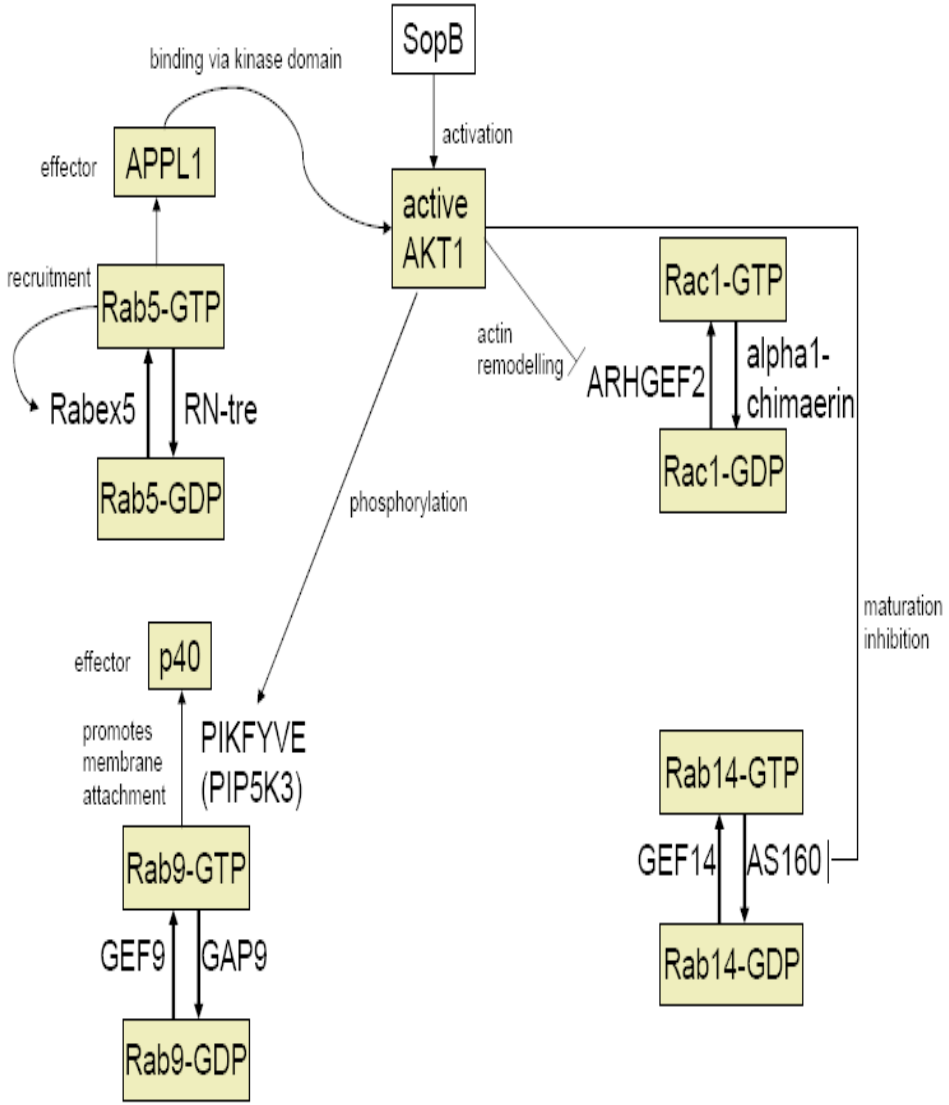
We have built mathematical models that concern active-inactive kinase-phosphatase complexes (and vice versa) and investigated the effect of these complexes on the shape of the dose-response curves. In particular we have found inactive kinase-active phosphatase case can lead to bump-like response curves, similar to the PIPs profiles in Yeung & Grinstein (2007). A manuscript is in preparation on this work.

Currently, our objective is to incorporate experimental data (already available in Dresden) into our PIP network regulated by Rab5. We have obtained EEA1 immunofluorescence data upon knock-down of the genes of MPI-CBG Rab5 effector library, which includes lipid kinases and phosphatases described by Shin et al (2005). Other data is expected from NKI-LUMC, to model how the *Salmonella typhimurium* effector SopB promotes intracellular survival by controlling actin dynamics and phagosome-lysosome fusion through the activation of AKT1 (Kuijl et al., 2007; see figures below).

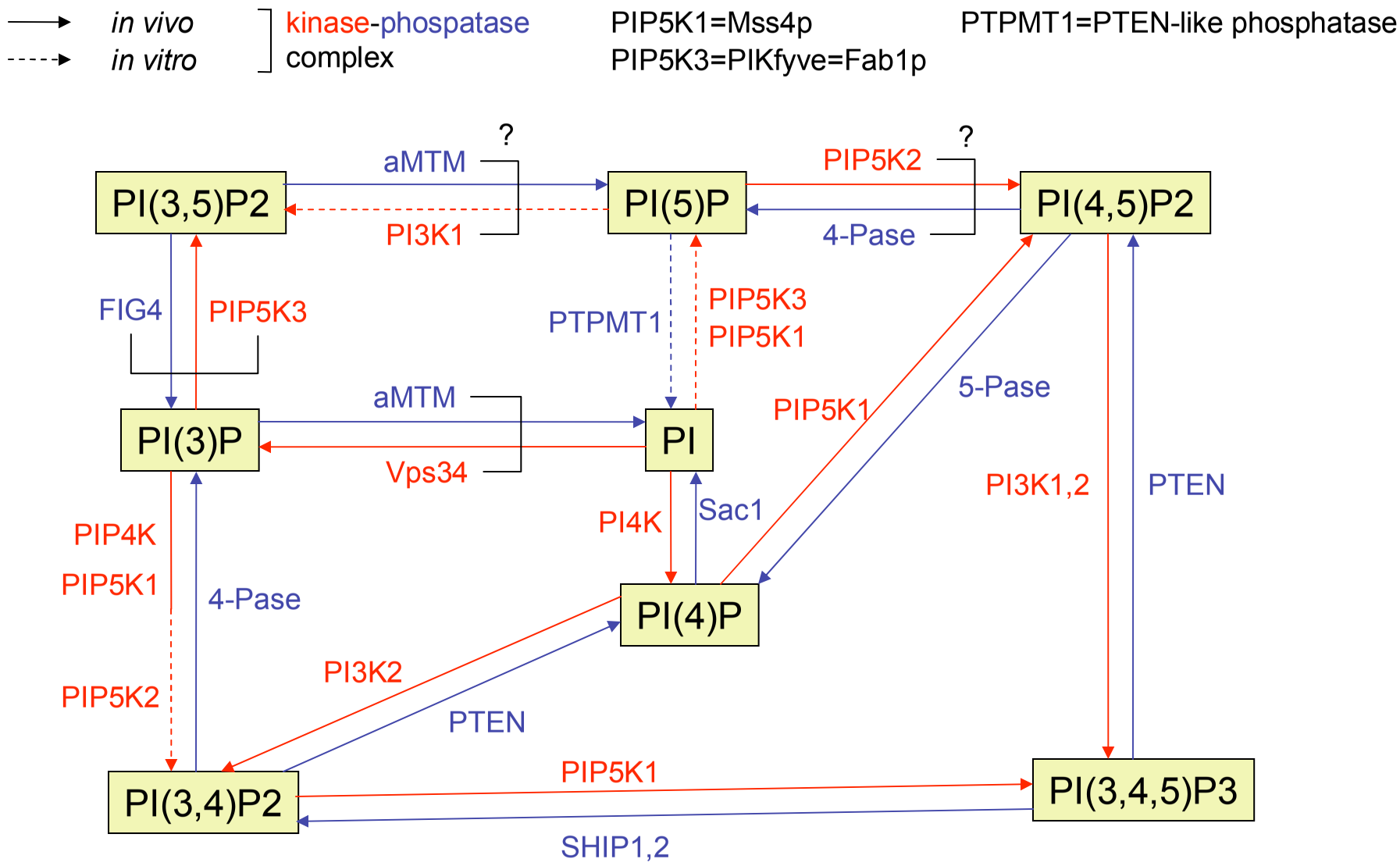
Two additional collaborations have been started as a consequence of this project. A collaboration with experimentalists at the London School of Hygiene and Tropical Medicine is planned in order to model how the lipids secreted by *M. tuberculosis* affect phagocytosis. Modelling input is being given on experimental design to address a number of questions that will help establish what interactions there are between mycobacterial lipids and PIPs. A further collaboration with JSI aims to re-construct the qualitative profiles of PIPs as published (Yeung & Grinstein, 2007), once we have identified the original data. A no-cost extension is requested to allow this work to be completed and some deliverables have been delayed accordingly (see management section for details).



PIPs involved in the Rab5 stage. Bold letters- knock-down genes from Dresden.



Salmonella effector SopB manipulates phagocytosis by phosphorylating Akt1. Data expected from Amsterdam.



Kinase-phosphatase pairs identified up-to-date, some are hypothetical (?).

Meanwhile, JSI has focussed on identifying the problems/weaknesses of automated modelling (AM) methods in the context of modelling phagocytosis and similar problems from systems biology. To this end, AM has been applied to reconstruct the model of endosome maturation (Rab5-Rab7) developed at the MPICBG, Dresden (del Conte-Zerial, 2008), both from simulated and measured data, as well as domain knowledge that we formulated based on the paper describing the model and its development (Todorovski and Džeroski 2009). Parameter fitting was identified as a major weakness of our methods and we have been working on developing improved methods for parameter fitting based on meta-heuristics (Tashkova et al, 2010).

Another relevant means for assessing the usability of AM methods in systems biology is the task of reconstructing a yeast synthetic network (Cantone et al, 2009), for which measured data and domain knowledge is available. We have formulated a library of domain knowledge and are currently working on applying AM methods to this problem. We expect that further strengths and weaknesses of our AM methods will be revealed in the process.

With the aim of a better understanding the process of phagocytosis, we have also developed other machine learning methods, such as predictive clustering trees for clustering time series data (Slavkov et al. 2010), and used them to analyse a variety of data related to phagocytosis of mycobacteria. In particular, we have used predictive clustering trees to analyse data on siRNA and compound screens of endocytosis in HeLa cells (data from MPICBG), a siRNA screen aimed at studying MHC ClassII antigen presentation (data from NKI), and time course profiles of gene expression level in response to infection for Type 1 and Type 2 human macrophages infected with *M. tuberculosis,* and Schwann cells infected with *M. leprae* (data from LUMC). These analyses are currently underway.