Modeling Stem Cell Differentiation into Dendritic Cells by

Systems Biology

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# Summary

This project proposal intends to meet challenges from different scientific disciplines: Biology, Computer Science and Modeling & Simulation Sciences.

The biological challenge is to identify the master regulators and their network, which drive cell fate decisions in blood stem cells towards highly specialized immune cells (dendritic cells, DC).

The challenge in computation, modeling and simulation is to generate a network-based model, which allows simulation and prediction of the process of cell fate decisions. The unique challenge here is to combine the short-term (hours) and long-term processes (days to 2-3 weeks) during this transition.

The project will provide a win-win situation, since the stem cell-DC system will be used as model system for the mathematical prediction of generic cell fate decision processes. Biology will obtain the information on master regulators and how they work in the network of stem cell differentiation. Computational and modeling sciences will develop a framework to link molecular and cellular dynamics. Such framework will close the gap between the short-term and long-term dynamics that exists in systems biology and thereby will open a broad range of future biomedical applications.

The project combines the expertise of junior (KS and IGC) and senior investigators (AS).

# Introduction: sketch of the biological system

Stem cells are unique biological entities, as they combine the capacity to self-renew with the ability to differentiate into various specialized cell types. This makes them very attractive research objects. Indeed, understanding the mechanisms that control either stem cell maintenance or stem cell differentiation, allows guiding and controlling these two important biological processes. Historically, the hematopoietic ‘organ’ is used as a model system to study stem cell biology and behavior1. The hematopoietic system consists of all cellular components found in blood. It thus includes platelets, red blood cells and white blood cells, which are important in hemostasis, oxygen transport and immunity, respectively, and which all develop from hematopoietic stem cells (HSC). Adult HSC are capable of maintaining the hematopoietic system life-long, a characteristic that forms the basis of, for example, stem cell transplantation as treatment of leukemic diseases.

Dendritic cells (DC) constitute a small, but very powerful population of white blood cells2. DC are the professional antigen presenting cells of our immune system. As such they take center stage in the immune response to self and non-self antigens and the subsequent induction of tolerance or immunity (Nobel prize medicine, 2011). DC are found in all organs, moreover, many types of DC exist, each with a specialized function, to protect our body optimally against the wide variety of invading pathogens. Deregulation of DC development or function is associated with many pathological conditions, ranging from autoimmune diseases, like psoriasis or rheumatoid arthritis, to allergies and immune deficiencies.

# Current State-of-the-Art

(1) Biology (KS)

Development of HSC into terminally differentiated cells is a multi-step process (Figure 1). First, HSC loose their stemness as they progress into multipotent progenitor cells (MPP), an intermediate, which still can generate all cells of the hematopoietic system1. Next, MPP restrict their potential and give rise to only one specific cell type, a process referred to as lineage restriction or commitment. When MPP commit to DC lineage, they progress into common dendritic progenitors (CDP) (Figure 1), which then differentiate further into various DC types, like classical and plasmacytoid DC (cDC and pDC, respectively)3.

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| **Figure 1: Hypothetical molecular network of transcription factors and their target genes in multipotent progenitors (MPP) and common dendritic progenitors (CDP). Plasmacytoid DC, pDC; classical DC, cDC. Please note different molecular interactions in MPP and CDP, left and right panel, respectively.** |

The identity of each of these cell states is determined by the sum of all genes that are expressed in that specific cell. Thus, with every transition from one cell state to the next, e.g. with transition from MPP to CDP, a conversion of gene expression occurs, ‘erasing’ the old identity (MPP) and imprinting the new one (CDP). Such transitions or cell fate decisions are directed by extracellular and intracellular components. Extracellular components are soluble factors present in the cell’s environment, like growth factors and cytokines. Intracellular components that guide lineage commitment are transcription factors that bind to gene-regulatory elements (enhancers and promoters) to directly steer gene expression.

Work with gene knockout mice has identified a panel of transcription factors that impact on various stages of hematopoiesis (Figure 1), for example some are critical for stem cells (e.g. Runx1, Tal1 and Gfi1), while others, like E2A, are specific for lymphocytes1. Similarly, factors were discovered that affect DC development, such as Pu.1, Id2, IRF8, SpiB, E2-2, Stat3 and Ikaros4. Via single gene analysis, many interactions (activating or inhibitory) and feedback loops have been identified. With increasing number of factors and interactions, complicated gene networks arise (Figure 1). The expression of cell type specific factors within such network will determine the cell’s gene expression repertoire. Since MPP are multipotent, they express factors of all hematopoietic lineages, as well as of stem cells (Figure 1, left box). When MPP progress to CDP and undergo DC-lineage commitment, expression of DC-associated transcription factors, such as Stat3, Id2 and IRF8, will increase (Figure 1, right box), which causes activation of the DC gene expression repertoire and inactivation of factors related to other blood cell lineages.

Recent evidence suggests that to establish a specific cell state and stably maintain it, only a few transcription factors – the master regulators – are needed5. Master regulators are at the center of the network, driving their own (autoregulatory) and each other’s expression, and regulating expression of the other genes of the network. There are indications that Pu.1 is an important regulator for all hematopoietic cells6. Pu.1 induces cell fate decisions by engaging lineage-determining factors (E2A for B cells or C/EBPa for phagocytic cells) to collaboratively occupy lineage-specific binding sites. Pu.1 binding then induces nucleosome/epigenetic remodeling (deposition of activating marks on histones), leading to expression of lineage-specific genes. Thus, Pu.1 might be central in the CDP network, as it induces expression of Flt3, a key growth factor receptor for DC development7. However, the DC lineage specifying factors are not identified yet.

Thanks to technological advances in the last decade, biology has passed the stage of single gene analysis. The use of microarrays, to monitor global gene expression analysis, is now further substantiated by genome-wide methodologies, like chromatin immunoprecipitation (ChIP) followed by high-throughput sequencing (ChIP-Seq). ChIP-Seq allows to elucidate the regulatory mechanisms of gene expression, via analysis of (i) transcription factor binding to gene-regulatory elements and (ii) the epigenetic status (= accessibility) of chromatin, defined by presence of e.g. activating or repressing histone marks. The use of genome-wide methods, however, has introduced another dimension of complexity. This complexity poses a limitation for biologists, as they can no longer interpret the vast amount of data and gain insight into biological processes, without the use of advanced computational tools.

Thus, the challenge in biology today is to take full advantage of the wealth of data generated (data files of 10 GB per sample, which mount to data sets of several hundred GB per experiment). This requires implementing integrated approaches to identify and model interactions of the biological entities that are involved in the processes of interest. This interdisciplinary challenge critically depends on establishment of close interconnections between Biology, Computer Science and Modeling & Simulation Sciences.

(2) Computational Regulatory Genomics (IGC)

The computational analysis of ChIP-Seq data is the current state of art for unraveling the molecular networks of transcription factors and their target genes on a genome-wide level. For example, ChIP-Seq data from 9 stem cell factors were used to uncover the regulatory networks of ES cells8. However, success of ChIP-Seq assays depends on the existence of a good antibody against the protein of interest and on availability of large numbers of cells, two conditions not always met. Therefore, ChIP-Seq-based studies are restricted to analysis of a few factors and few cell types8-10.

An alternative to overcome these limitations is the use of traditional, sequence-based bioinformatics methods, which search over the genome for motifs representing the DNA binding sequence of a transcription factor. However, motifs are generally small and with low binding specificity, and moreover, the presence of a motif does not imply actual transcription factor binding in that particular cell. As a result, motif-based methods return hundred thousands of putative binding sites, of which only a small fraction is actually active in a particular cell type. Recent studies explored the fact that an open chromatin structure is crucial and pre-requisite to the binding of a transcription factor to a particular DNA sequence11. Open chromatin state can be measured directly on a genome wide scale with DNase I sequencing (DNase-Seq) experiments12. Alternatively, it can be inferred from the presence of activating histone modifications, measured by ChIP-Seq, such as H3k4me3, H3k4me1 and H3k27ac11 or from the vicinity of binding sites for master regulators13. Recent bioinformatics research showed that DNase-Seq12 and the combination of DNase-Seq and histone modifications14 can greatly improve the accuracy of motif search methods. Furthermore, it allows detection of cell specific and combinatorial binding sites.

Such approaches open up new possibilities to detect cell-specific binding sites for all factors with known motifs, starting from only a small number of ChIP-Seq experiments per cell of interest. So far, most studies using open chromatin information to improve motif searching have analyzed single or mostly unrelated cell lines12,14,15. A current open challenge, therefore, is the use of inference of such networks during a dynamical biological process, such as cell differentiation, and the use of mathematical modeling methods for the conceptual validation of these networks.

(3) Modeling (AS)

Computational data analysis and predictive modeling of cell fate decisions and their induction by the biological environment, is one of the most pressing needs in systems biology. In the last decade, systems biology has tried to establish such models, however, a breakthrough towards biomedical applications could not be achieved so far16. Systems biology research mostly concentrated on modeling of signal transduction, which typically covers short time scales of minutes and hours, by using ordinary differential equations. Long-term processes, like development of cell populations, which take days to weeks, have been modeled using population dynamics models. These models quantify the dynamics of and mutual transitions between cell populations, which is characterized by the emergence of phenotypically different subpopulations17,18. The approaches used so far suffer from the lack of knowledge about the link between (i) models on mechanistic, sub-cellular short-term processes and (ii) population models describing long-term processes. Today, this lack is recognized as the major bottleneck for efficient prediction of cell fate decision processes, based on data derived from biological experimentation.

Therefore, the challenge is to develop an efficient data analysis and modeling approach to assess and quantify the mechanisms which play (i) a key role in the local molecular induction of cellular processes and (ii) induce long-term transformations of the cellular phenotype, involving cellular mechanisms with thousands of genes, frequently referred to as cellular plasticity. Although first promising results have been reported recently19, an integrated concept for efficient reengineering of the relevant mechanisms, based on biological data, is not available today.

# Relevant Work of the Applicants

(1) Seré

Our research aims at unraveling the molecular mechanisms, which underlie stem cell differentiation and lineage commitment from MPP to CDP and finally into DC20. By comparing genome-wide gene expression microarray data of MPP and CDP, we found that CDP exhibit a DC-primed expression pattern and we nailed down the DC-lineage associated transcription factors20,21. A set of selected, promising factors was (and is) studied in more detail, by use of gene knockout mice. We eludidated for example, that Id2 is needed for DC development under steady state conditions, but not during inflammation22. We also revealed that IRF8 is key to DC commitment, as (i) IRF8 deficient MPP do not develop into DC, and (ii) IRF8 overexpression induces a CDP-like expression pattern23 (Seré et al., in preparation).

We extended our studies to the epigenetic changes during MPP to CDP transition, using ChIP-PCR or ChIP-Seq for activating and repressing histone marks (H3K4me3, H3K4me27, H3K9me3 and H3K9Ac). We discovered that the transition from MPP to CDP is accompanied by a profound rearrangement of histone marks, which parallels the changes in gene expression (Lin et al., in preparation). Intriguingly, ChIP-Seq for Pu.1 – a potential master regulator of DC lineage – on MPP and CDP also showed a prominent conversion of Pu.1 binding sites. When MPP progress to CDP, strong Pu.1 binding is gained at enhancer and promoter of DC lineage genes. Currently, we investigate potential binding partners of Pu.1.

(2) Costa

Costa Lab develops computational biology approaches for integrated analysis of genome-wide gene expression data, histone modifications (ChIP-Seq), open chromatin assay (DNase-Seq) and transcription factor binding sites. Initial work was the establishment of a method to visualize, query and detect modules of co-regulation genes during blood cell development24,25. Next, we developed a method, based on sparse linear regression estimation, to predict the regulatory roles of transcription factors and histone modifications during blood cell development26,27. Using a hidden Markov Model-based methodology, we simultaneously analyzed histone modifications (ChIP-Seq) and open chromatin (DNase-Seq) to predict cell-specific transcription factor binding sites14.

(3) Schuppert

In the last years, novel methods to tackle the analysis and modeling of cellular plasticity have been developed at AICES (Schuppert lab). By establishing efficient and highly specific cell characterization, based on full-genome expression data28, as well as on epigenetic methylation data (Schuldt et al., in preparation), cell fate characterization and tracking of induced cellular plasticity could be significantly improved. Moreover, a quantitative link between expression data from cellular transformation experiments on the lab scale and clinical data derived from publicly available data repositories in a “big data” approach29 (Schuldt et al., in preparation) could be established. This was used for tracking of induced cellular dynamics in single cell experimentation30.

To link induced cellular transformation and environmental stress, a network reconstruction method on a full-genome scale was developed, which was used to describe the response of cells to toxic compounds31. This approach suggested that intrinsic co-regulation allows generic genome-wide reprogramming using not more than 5-15 appropriate induction factors32. In order to characterize the mechanistic networks relevant for reprogramming of cells on the molecular level a mathematical framework has been described33. This framework was applied to analyse the mechanisms that lead to drug resistance in leukemic cells34. In collaboration with Prof. Alexander Mitsos, Chair for Systems Technology at RWTH Aachen University, an algorithm for detailed reengineering of cellular networks has been established at AICES.

# Goals and Approach (Methodology)

DC are nowadays appreciated as crucially important immune cells with great clinical potential. However, the understanding of their development from HSC and MPP remains a blind spot in hematopoiesis: still, DC are ill-positioned – if positioned at all – on the hematopoietic roadmaps. This gap is caused by the lack of knowledge about the factors, and their molecular mechanisms, that drive cell fate decisions towards DC lineage.

In this project, therefore, we intend to establish a network-based model, which allows simulation and prediction of the time course of HSC differentiation in general, and DC lineage commitment from MPP to CDP in particular. The heterogeneity of the time scales of the relevant sub-processes (short-term processes like signal transduction [hours] versus long-term processes like changes in cellular phenotype [days to 2-3 weeks]) will require the development of novel, multi-scale modeling approaches to cover the relevant dynamic processes correctly. Stochastic transitions between steady-states can be realized by transition state models using Markov networks. However, recent publications35 indicate that the initiation of stem cell differentiation cannot be described by stationary states. Experimental results suggest that nonlinear dynamic structures, such as limit cycles, play a crucial role in stem cell differentiation. It is assumed that the dynamics can be described by the set of intrinsic attractive states of the cellular regulation network, comprising negative and positive feedback loops.

# Working Plan

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| **Figure 2: Schematic overview of the workflow, including the biological (a), regulatory genomics (b), modeling (c) and validation steps (d).** |

The model to be developed has to integrate two heterogeneous techniques in a novel approach:

- In a first step, the Schuppert group will apply mechanistic modeling, starting from a Boolean network, for representation of the underlying regulatory network at the molecular level, on genome-wide expression data, provided by the Seré group (Figure 2a). In order to identify the stable steady states, the transition states as well as the stable limit cycles of the established signaling network, we will use markov-chain monte carlo techniques. A significant challenge will be the fit of the simulation data to experimental data, which will be an ill-posed inverse problem. Additionally, the Costa group will apply computational methodologies for uncovering the regulatory network during DC development. The analysis will be based on gene expression and ChIP-Seq data from histone modifications and the hematopoietic master regulator Pu.1 (Figure 2b). This will reduce the ambiguity of the networks normally inferred from data. For further improvement of stability, we will reduce the dimensionality by appropriate projection on subspaces spanned by genome-wide expression patterns36. In addition, we will analyze whether compressed sensing will be sufficient to identify the relevant biological mechanisms.

- In a second step, we will relate the identified network dynamics on the molecular scale to the phenotypical transition model of MPP progression into CDP, using a transition state approach by ergodic averaging of the dynamics. This reduction of the dynamic complexity on the molecular scale to a simple model on the macroscopic scale is critical for establishing the linkage between mechanistic dynamic molecular models and macroscopic cell development (MPP to CDP transition). Establishing an efficient averaging procedure will close the gap between short-term molecular mechanisms and long-term processes (Figure 2c).

- To complement the abovementioned semi-mechanistic multi-scale models, we will apply our recently established PhysioSpace method to map the identified stem cell differentiation dynamics onto a genome-wide background. Initial data suggest that this approach can be successfully applied to stem cell differentiation experiments (AS and B. Schuldt, unpublished). In this project, we will for the first time relate the molecular dynamics via gene-set enrichment and pathway-enrichment to the observed genome-wide cell dynamics.

- Establishment of the described network-based model allows to predict the outcome of biological experiments, for example the application of environmental stress to a cell (e.g. inflammation or drug treatment) or the effect of gene dosage on cell differentiation (i.e. gene knockout or overexpression). These predictions will be validated by retrospective analysis of publicly available data and by experiments using in vivo and in vitro biological model systems (Figure 2d).

# Financial Plan

Given the highly specialized modeling work and the limited time frame, the main part of the funding will be a 1-year post-doc position (60.000 Euro). For the biological validation (microarray analysis, ChIP-Seq, in vitro cell culture, work with knockout mice) we request an additional 20.000 Euro (growth factors, tissue culture medium, Affymetrix GeneChip arrays, antibodies for ChIP). The costs for a technician performing the biological validation will be covered by institutional funding.

# Expected Long Term Impact

Integrating the three modeling steps described above, we aim to establish for the first time a closed model framework, linking molecular dynamics and macroscopic cell dynamics. Hematopoietic cell fate decisions provide an ideal system for conceptual model development, as the underlying dynamical features dominate drug efficacy, toxicology or even induced stress tolerance in plants. Although our model system appears to be simple enough to allow a proof of concept throughout the project, it provides perfect options for dissemination into a broad range of application areas.

Computational Biomedicine is a rapidly arising area of research at RWTH Aachen University. It integrates the strengths in (i) Modeling & Simulation Sciences e.g. at the Center for Computational Engineering Science (CCES) and the Aachen Institute for Advanced Study in Computational Engineering Sciences (AICES), (ii) the newly established IZKF Computational Biology Research group and (iii) biomedical research in stem cell biology, genomics and systems biology. Hence, it brings together the engineering and biomedical competences at RWTH Aachen University, which perfectly fits to the long-term strategies of both RWTH Aachen University and its Medical School.

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