Analysis of cellular pedigrees - A working report

Katrin Braesel (research fellow)

Ingmar Glauche (PhD student)

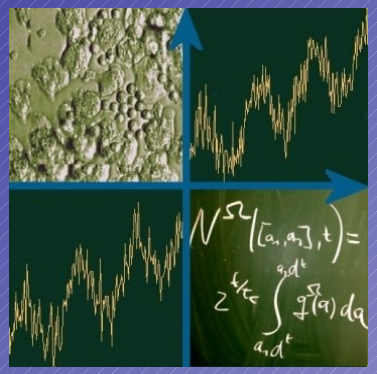
Matthias Horn (Diploma student)

Prof. M. Loeffler (Institute director)

Ronny Lorenz (trainee)

Christian Luecke (MD student)

Dr. Ingo Roeder (group leader)



Project Group:

DYNAmical MOdeling of Tissue Stem Cell Organization

Institute for Medical Informatics, Statistics and Epidemiology

University of Leipzig

Biological Background

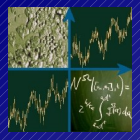
Stem cells of a particular tissue are

- **a (potentially heterogeneous) population of functionally undifferentiated cells**
- **capable of:**
  - homing to an appropriate growth-environment
  - proliferation
  - production of large numbers of differentiated progeny
  - self-renewing or self-maintaining their population
  - regenerating functional tissue after injury

(Definition of tissue stem cells [Loeffler and Roeder, 2002])

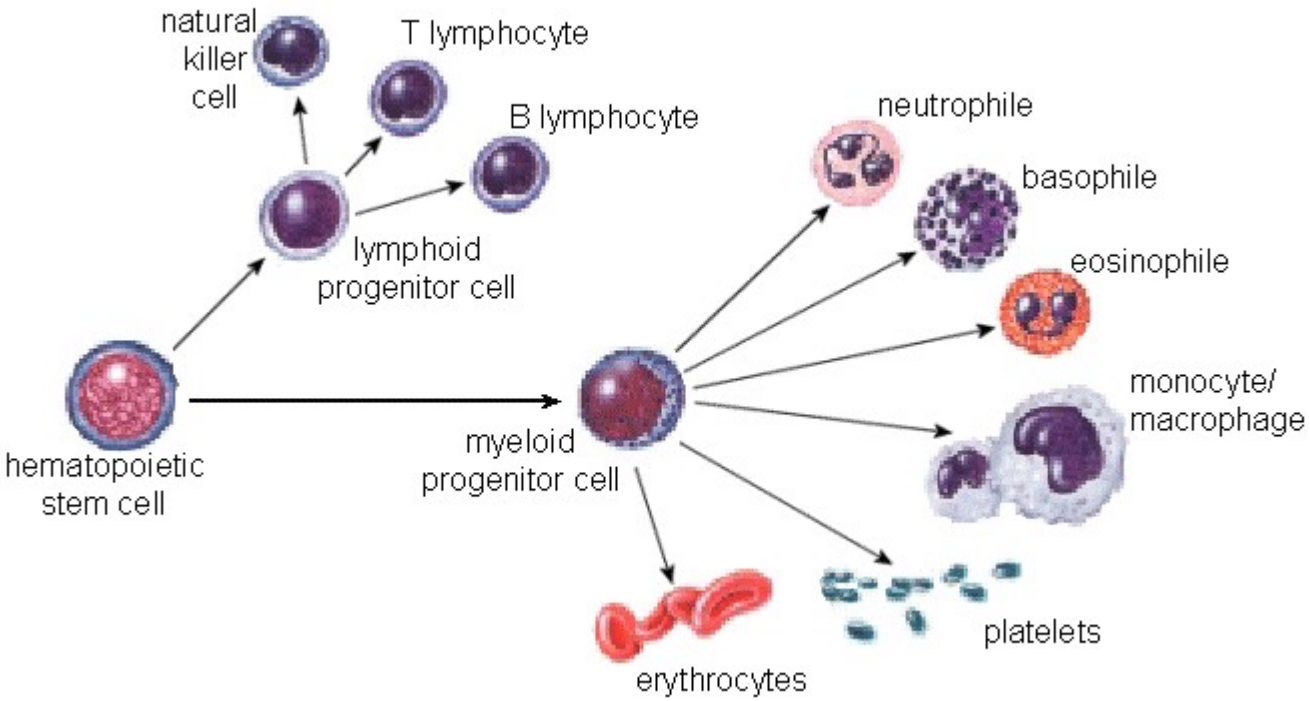
Hematopoietic stem cells (HSC's)

- one kind of somatic tissue stem cells
- located mainly in the bone marrow
- precursors for all blood and immune cells
- most investigated tissue stem cell system (since 1960's)



Biological Background

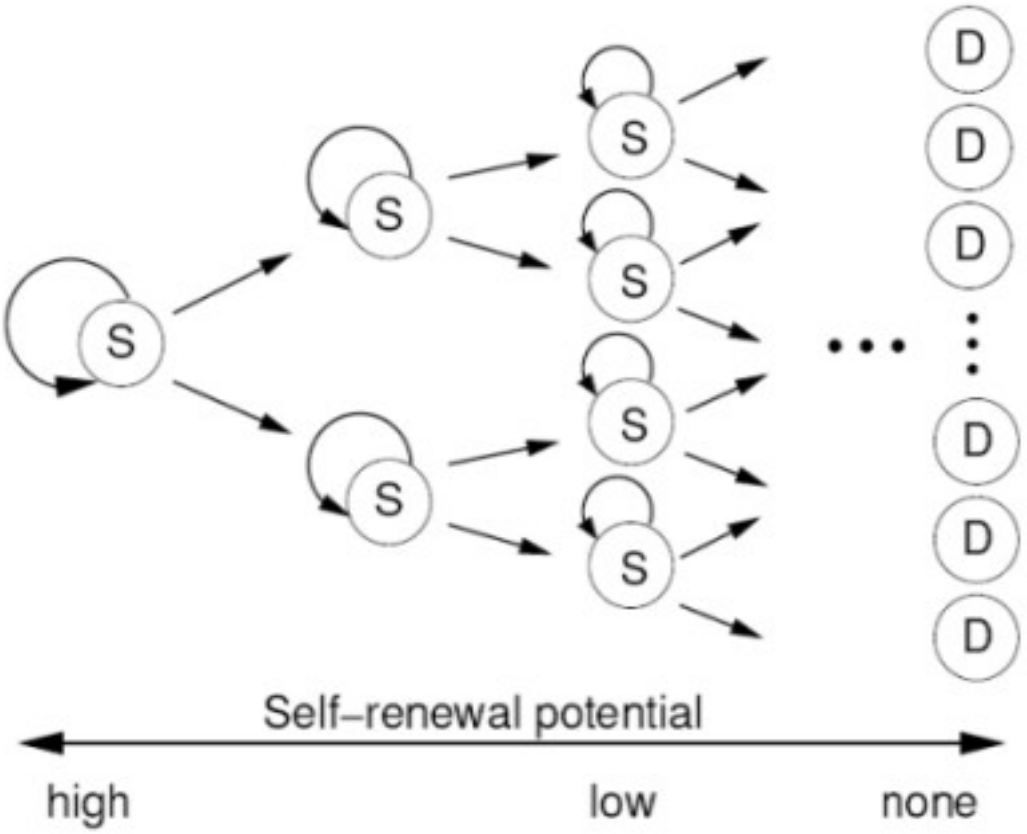
HSC differentiation scheme:



Biological Background

HSC within hierarchy:

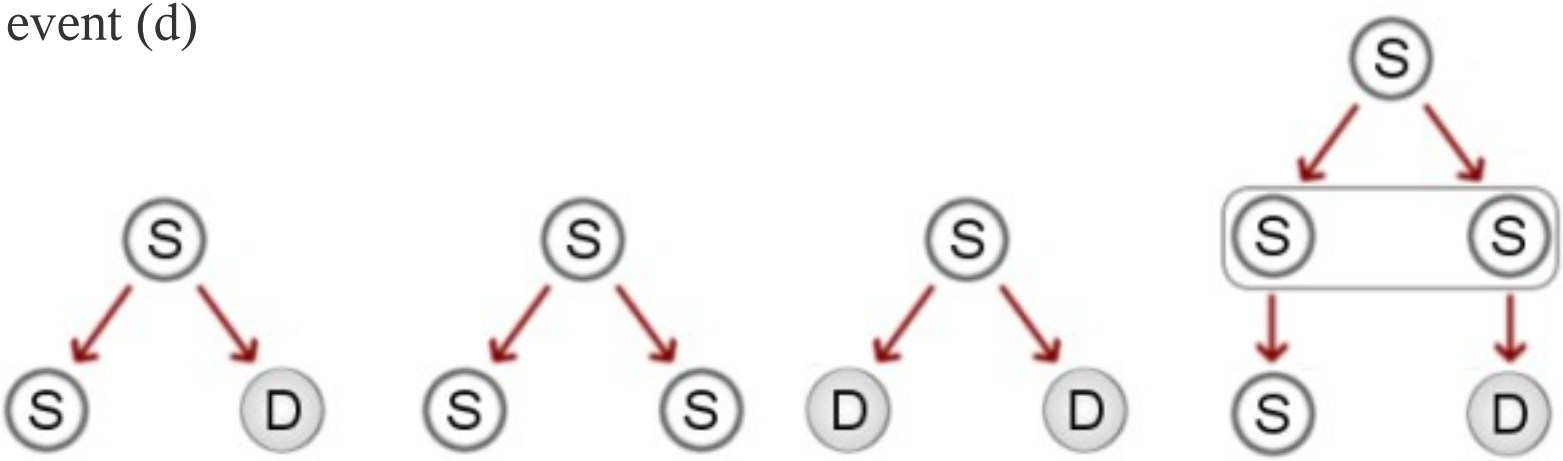
- self-renewal potential of stem cells (S) is gradually lost
- differentiated cells (D) are assumed to have lost self-renewing potential completely
- Is there a regaining of self-renewing potential?
- heavily debated due to observed plasticity and flexibility of HSC's in experimental work



Biological Background

Suggested types of stem-cell division:

- asymmetric division into one stem cell (S) and one differentiated daughter cell (D) [Till et al., 1964; Ogawa and Mosmannn, 1985]
  – no self-renewing potential (a)
- symmetric divisions into two identical stem cells or two differentiated cells [Vogel et al., 1969; Loeffler and Grossmann, 1991]
  – change of rates for these two types induces growth/reproduction of stem cell population (b) & (c)
- symmetric reproduction followed by an independent differentiation event (d)



(a) (b) (c) (d)

Asymmetric/Symmetric division 1

Asymm. Division:

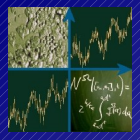
- unequal distribution of cell-content during division

Symm. Division:

- unequal cell-development after division

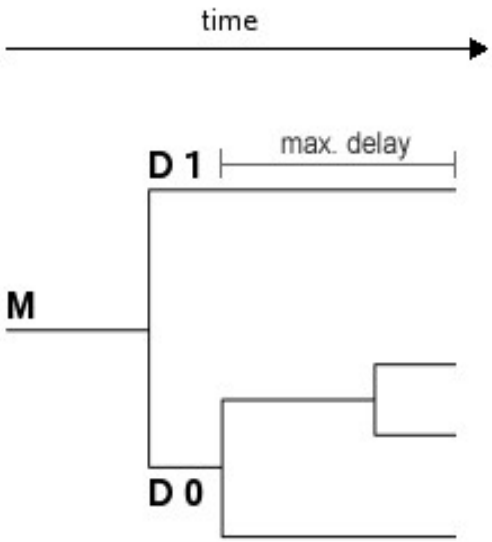
Is asymmetric stem cell division necessary to explain asymmetric stem cell fate?

- at the moment there is no evidence for asymmetric cell division in the hematopoietic system
- asymmetric stem cell fate often interpreted as asymmetric stem cell division
- different possibilities to define stem cell fate, with respect to:
  - lineage commitment
  - cycling activity
  - apoptosis
  - ...

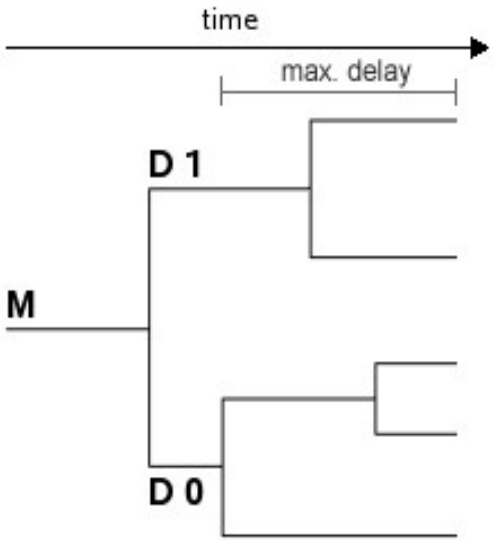


Asymmetric/Symmetric division 2

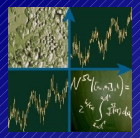
We took a closer look at the second type:



asymmetric



symmetric



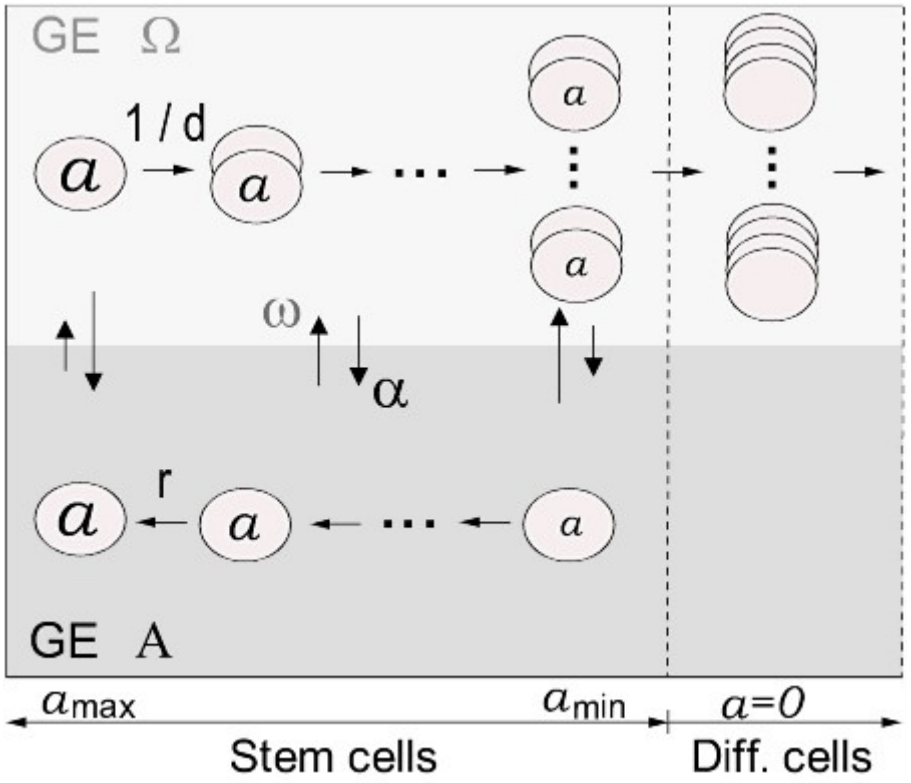
Model - description

Type and properties of the model

- stochastic single cell based model
- cells are updated at specific timepoints according to predefined rules
- no assumption of unidirectional stem cell hierarchy necessary to explain self-renewal/self-maintenance and plasticity

In (more or less) detail:

- 2 growth environments GE A, GE Ω
- affinity *a* for attachment to GE A, indicates potential of stem cell
- proliferation only in GE Ω, fixed average turnover-time
- **all cell divisions are symmetric**



Simulation strategy

Experimental data: (Punzel et al./Experimental Hematology 31 (2003))

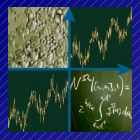
- in-vitro culture of hematopoietic stem cells without stroma support
- observation time of 10 days
- 13 x 96 single cell observations every 12-24h on “96-well plates”

Model assumptions:

- parameter set for in-vitro mouse experiments determined in former DYNAMO-projects
- simulation time of 10 days
- average cell cycle turnover was set to 24h

Simulation procedure:

- variable initial *a* value for each single cell
- 100 x 96 simulation runs
- calculation of asymmetric division rate (AD)



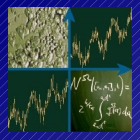
24h cct / 10days simulation time

First simulation results:

- experimental AD: 22.8%
- simulated AD: 11.2%

Why is there such a difference?

- nothing known about experimental observed cell cycle times
- fewer cell divisions in experiment than 24h cct produces



Different cellcycle times

Results with 48h cct/10d simulation time:

- experimental AD: 22.8%
- simulated AD: 13.3%

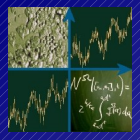
Results with 60h cct/10d simulation time:

- experimental AD 22.8%
- simulated AD: 14.1%

Results with 72h cct/10d simulation time:

- experimental AD: 22.8%
- simulated AD: 13.8%

Effect can not only depend on cell cycle time...



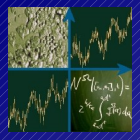
24h cct / 6days simulation time

Suggested solution:

- it has been shown, that stem cells are irritated for some time after they are placed in strange in-vitro conditions
- no cell cycle activity in this time
- we assume:
  - 4 days of delay to regard initial irritation
  - regular 24h of cell cycle time

Results with 24cct/6d simulation time:

- experimental AD: 22.8%
- in-silico produced AD: 20.9%



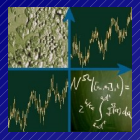
Conclusion 1

Summary:

- no special fitting of parameter set
- no need for asymmetric division of stem cells
- further simulation sets in progress

Problems:

- How is the distribution of cell cycle time?
- How long is the initial delay due to irritation?

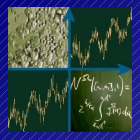


Conclusion 2

Analysis and Comparison with experimental cell tracking:

- experimental based pedigrees from single cell tracking in progress (done by Timm Schroeder, GSF - National Research Center for Environment and Health)
- not only for divisional history but for much more hematopoietic stem cell research topics

Open question:



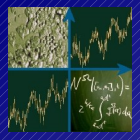
Conclusion 2

Analysis and Comparison with experimental cell tracking:

- experimental based pedigrees from single cell tracking in progress (done by Timm Schroeder, GSF - National Research Center for Environment and Health)
- not only for divisional history but for much more hematopoietic stem cell research topics

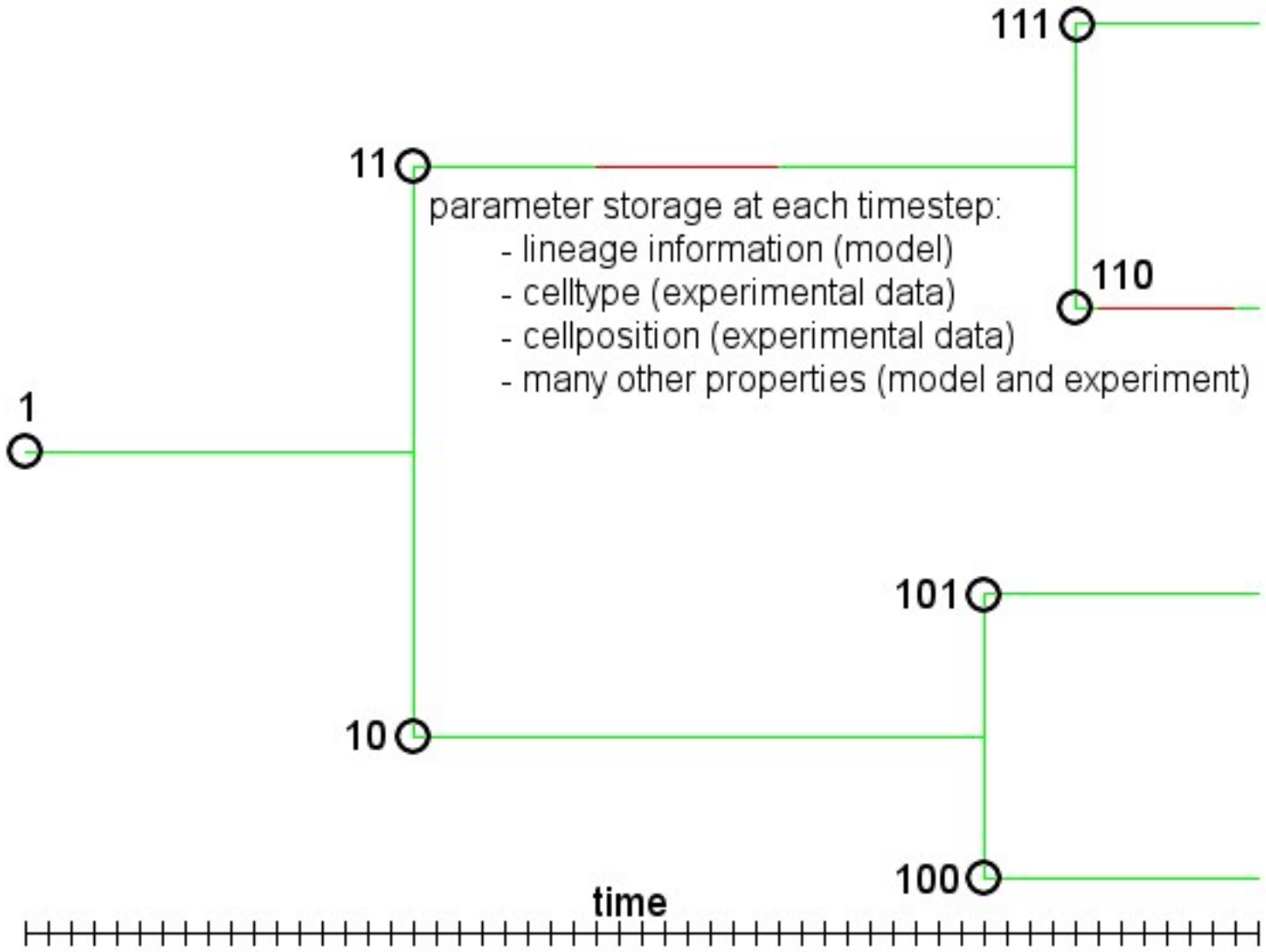
Open question:

How to compare and analyse complex binary, weighted trees?



Our pedigrees are complex

An example tree:



Open questions

Comparison of experimental trees

Comparison of experimental trees with in-silico trees

- What mechanisms play an important role in the model/biological system?

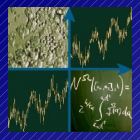
Central Question

How to compare trees?

...with respect to:

- distribution of cell cycle time
- occurrence of apoptosis
- lineage commitment
- velocity of the cells
- ...

We are open to any ideas!



Open questions

Comparison of experimental trees

Comparison of experimental trees with in-silico trees

- What mechanisms play an important role in the model/biological system?

Central Question

How to compare trees?

...with respect to:

- distribution of cell cycle time
- occurrence of apoptosis
- lineage commitment
- velocity of the cells
- ...

We are open to any ideas!

Thank You!