Towards the modelling of the regulation of early haematopoiesis

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Abstract

Haematopoietic stem cells (HSCs) can either remain quiescent, with a constant and low rate of self renewal, or become committed to differentiation into haematopoietic cell lineages. The switch between those two fates can be seen as the switch from one stationary state to another in a dynamic process (epigenetic switch). The control of early haematopoiesis is quite complex, involving several factors and feedback circuits. Its study needs modelling, which was not straightforward, since it requires to take into account both consumption and production of resources as well as discrete and continuous time. This was performed using Hybrid Functional Petri Nets (HFPN). The model presented simulates the results obtained *in vitro*, where the quiescent stem cells are rapidly lost, and shows the potentiality of HFPN to model complex systems such as the regulation of haematopoiesis.
In silico simulations can then be performed to study the mechanism responsible for the epigenetic switch.

Key words: haematopoiesis, regulation, cytokine, multi-scale modelling, Hybrid Petri nets, simulation.

1 Introduction

Haematopoiesis is a complex phenomenon leading to the continuous production of all types of mature blood cells. This process is ensured by a population of haematopoietic stem cells (HSCs), which are able by a process called self-renewal to maintain a constant pool *in vivo*. Nevertheless, HSCs do not self-renew *in vitro* despite the development of numerous methods of culture.

The proliferation and the differentiation of HSCs are greatly influenced by cytokines secreted by the cellular micro-environment, suggesting that paracrine loops are essentials in the haematopoietic cell production. Recently, a few evidences indicate that HSCs could control their own determination, by secreting some of their growth factors [**?**]. This constitutes the prerequisite element for formation of autocrine loops, which could be responsible for bi-stability of the HSCs.

Many molecules have been studied (unsuccessfully so far) as candidate autocrine factors, but the role of a cytokine, the interleukin-6 (IL-6), has not yet been considered. IL-6 associated with its cognate receptor (sIL-6R) could be involved in an autocrine feedback. We hypothesise that a potential feedback circuit in the IL-6 network could be responsible for the switch of HSCs between self-renewal and differentiation. In this way, IL-6 activation of gp130 can be considered as the leading step of this epigenetic switch [**?**].

The nature of the haematopoietic system gives hard some experiments. Consequently, it is advisable to first test this hypothesis *in silico*. Thus, we needed first to built an accurate model simulating the regulation of IL-6 during early haematopoiesis. In order to describe this process, several aspects must be considered, such as: cellular dynamics, since a cell can be subject to different evolutions during its life-time, molecular interactions, influences of the interleukins on the cells and *vice versa*.

To model all these aspects, we need a formalism which integrates the notion of production and consumption of resources (for modelling cellular and molecular variation). Moreover, the notions of

both discrete and continuous modelling are necessary to model respectively cellular and molecular interactions and evolutions. Among the formalisms existing in the literature, we focused on Hybrid Functional Petri Nets (HFPN) [?] [?]. These nets can merge all the required elements and they also allow to define functions. They are composed of places, transitions and arcs which can be either discrete or continuous.

2 Biological process

The regulation of HSCs involves numerous growth factors. We focused on IL-6 and the molecules directly associated, since this signalling pathway is known to play a central role in stem cell biology [?]. Receptors involved in recognition of IL-6 are IL-6R and gp130. The assembly of the complete signalling receptor is sequential and hierarchically ordered. IL-6R binds IL-6. IL-6R exists in two forms: a membrane-bound form, called mIL-6R, and a freely-soluble-form, called sIL-6R. The soluble form of the receptor, sIL-6R, is secreted in an autocrine fashion by HSCs.

The complexes IL-6/mIL-6R or IL-6/sIL-6R are recruited by two gp130 subunits (membrane receptor) and the resulting gp130/IL-6/IL-6R complexes are internalized. Gp130 stimulation triggers activation of several intra-cellular pathways, particularly JAK *(Janus kinase)*, which subsequently activates the STAT pathway *(Signal transducer and Activators of Transcription)* [?]. Once the JAK/STAT pathway is activated, it stimulates self-renewal as well as synthesis of the membrane receptors which were internalized (gp130 and mIL-6R). A part of mIL-6R is cleaved in the freely-soluble-form of the receptor. Finally, the JAK/STAT pathway activates SOCS proteins *(Suppressor of Cytokine Signaling)*. These SOCS act as an inhibitor of the JAK/STAT pathway.

Since the regulation of early haematopoiesis by IL-6 involves numerous and complex signalling pathways, it is indispensable to study this process *in silico*, before performing costly experiments.

3 Modelling by HFPN the role of IL-6R in early haematopoiesis regulation

The model presented Figure **??** simulates the role of interleukin-6 in early haematopoisis. This model was built in several steps corresponding to the multiple and interdependent regulation networks that are contitutive of the complex biological process of hematopoieis.

Cellular model

First, we built a sub-model for the cellular evolution which represents evolution of each cell lineage as a function of time. This sub-model is built in discrete time, since cells can be numbered (sub-model in the bottom part of Figure **??**). It contains three discrete places (symbolized by simple circles) representing three biological entities: quiescent stem cells *Pq*, permissive stem cells *Pp*, and cells committed to differentiation *C*. Discrete transitions (symbolized by filled rectangles) represent all the processes that allow a cell to change its state. A *Pp* cell can: leave the cell-cycle to become a *Pq* cell, and inversely (respectively, transitions *T2* and *T1*), symmetrically divide (self-renewal *T3* or differentiation *T6*), asymmetrically divide *(T4)*, and differentiate without division *(T5)*.

Molecular model

The second sub-model is the molecular model representing interactions between cytokines, i.e. association and dissociation of the molecular complexes. Since the formation and dissociation of complexes are continuous phenomena in cells, this sub-model was built in continuous time (top part of Figure **??**).

Each cytokine as well as each complex of interest is modelled by a continuous place (symbolized by double circles). We therefore have places for the molecules IL-6, sIL-6R, mIL-6R, gp130, IL-6/IL-6R and gp130/IL-6/IL-6R. The continuous transitions used in the model (symbolized by

empty rectangles) represent biological processes such as formation (*T7, T8, T9* and *T10*) and dissociation (*T11, T12* and *T13*) of all the complexes.

The final model (presented on Figure **??**) was obtained by modelling biological links between the two submodels (molecular interactions and cellular evolution), such as the autocrine secretion of sIL-6R by *Pp* cells *(T14)*, as well as the kinetics of signalling pathway *(Pv_1-Pv_6, T17-T20)*.

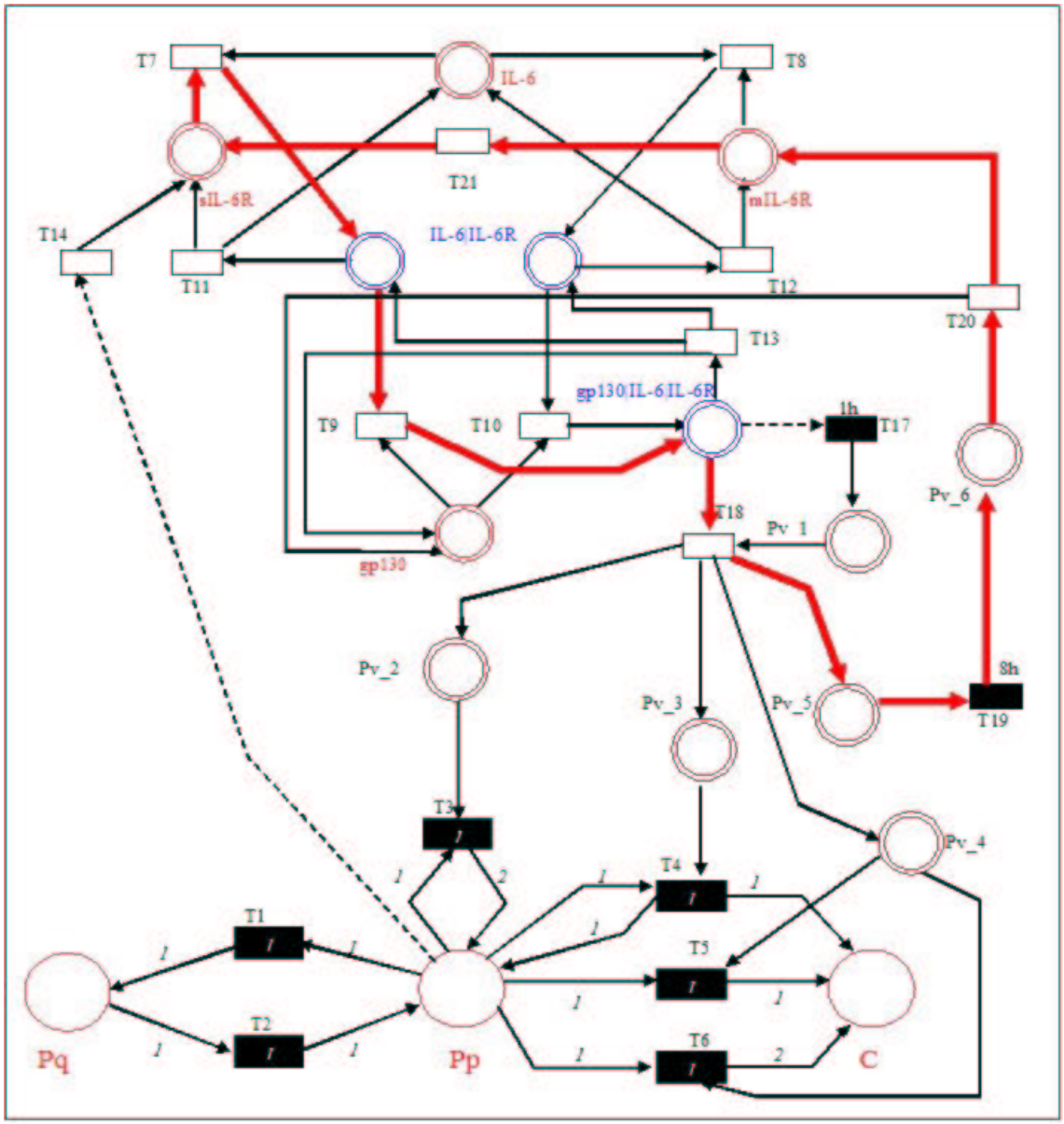

Figure 1: ***Global model.*** The cellular and molecular sub-models were joined thanks to biological links: the autocrine secretion of sIL-6R by permissive cells *(T14)* and the kinetics of signalling pathway *(Pv_1-Pv_6, T17-T20)*. The hypothetical feedback circuit is indicated in boldface arcs.

4 Results and discussion

Once the appropriate model of the IL-6-mediated regulation of the early haematopoiesis was built, a set of simulations was carried out with experimental values [**?**]. The simulations were done using the *Cell Illustrator* software [**?**] [**?**], which implements the HFPN formalism.

We concentrated our work on the study of the evolution of permissive cells (Pp) as a function of time. The simulation of the model led to the disappearance of permissive cells in about ten days (Figure **??**). This experiment subsequently tested *in vitro*, and the *in vitro* results conform to the *in silico* results [**?**]. Consequently, our model is adequate for further testing our hypothesis of an epigenetic switch.

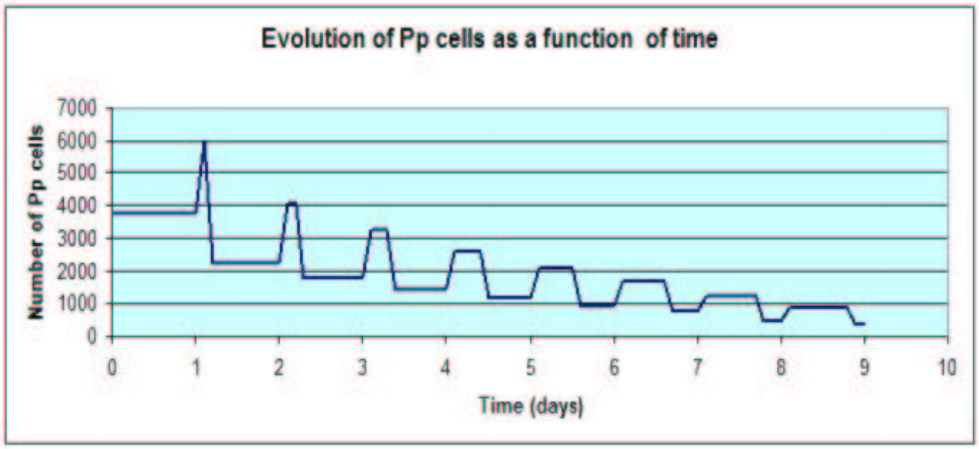

Figure 2: ***Evolution of Pp cells as a function of time.***

5 Conclusion

Haematopoiesis is a complex process involving numerous interdependent circuits in its regulation. To model such a system, we needed integrating different formalisms. The Hybrid Functional Petri Nets (HFPN) are Petri Nets which offer a maximum of functionalities. Results of simulations led to the disappearance of primitive HSC subpopulation. We succeeded to build a model, whose simulation results are in accordance with the *in vitro* results. Simulations can then be performed, in a future work, to study the mechanism responsible for the epigenetic switch.

The agreement of *in silico* and *in vitro* results provides informal validation of our model. Nevertheless, it would be interesting to validate a model in a more formal manner. At present, HFPN only enable simulations. We are therefore interested in developing a system of validation and verification for hybrid Petri nets considering a maximum of functionalities.

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