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From Bio-Inspired to Institutional-Inspired Collective Robotics

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Task 1: Epigenetic mechanisms controlling collective configurations in simulated Th cell populations

Progress Report

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FCT
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1 Introduction

The T helper (Th) cells have been extremely successful during the course of evolution as evidenced by their presence in all jawed vertebrate species. Central to their success is the important role they play in establishing and maximising the capabilities of the immune system. The Th cells are essential in directing the activation and growth of cytotoxic T cells, determining the antibody class switching for B cells, and maximising the bactericidal activity of phagocytes such as macrophages (Janeway et al., 1997). As a population, Th cells are capable of dynamically regulating and differentiating themselves into different sublineages (e.g. Th1, Th2, Th17, and Treg cells) (Harrington et al., 2005; Hori et al., 2003; Park et al., 2005; Mosmann and Coffman, 1989) to initiate the appropriate immune response.

The advent of modern systems biology has led to considerable research on the genetic regulatory networks (GRN) underlying individual T helper cells. Interactions between genes at the cellular levels have been represented with a variety of qualitative tools (e.g., Logical Networks and Petri Nets) (Naldi et al., 2010; Garg et al., 2009; Mendoza, 2006; Garg et al., 2007). These tools are particularly appealing because the precise role of different molecular species influencing Th cell differentiation is not know. In addition, qualitative tools may allow a better understanding of the stable states (cell sublineages) reached by the GRN of the Th cell. In a first move toward this goal, Naldi et al. (2010) developed a comprehensive logical network model of the signalling pathways underlying Th cell differentiation. Using this network, the authors highlighted the context-dependent stable states reached by the GRN of the Th cell, deduced from initial states and environmental conditions.

An important consideration of most of these models is that an individual Th cell is analysed independent of its environment. However, the interesting properties of the dynamics of the immune responses lie not only in the states of the individual cells, but also in their interactions with neighbouring cells i.e., the coupling of the GRN. Despite the advances made at the individual cell level, the interactions between cellular networks within a multi-cellular system has not yet been investigated systematically.

In this study we investigate how a coupling of GRN across a large number of peripheral Th cells (outside of the thymus) collectively influence the state of the population and consequently the characteristics of individual cells. We used an agent-based simulator to model a situation where individual Th cells were cabable of differentiating into various lineages. The logical network of Naldi et al. (2010) was used to model the signalling pathways underlying cell differentiation. Interactions between Th cells was made possible through shared conjugations at Antigent Presenting Cells (APCs). In addition, cells could influence their intermediate environment through the production and consumption of 13 different cytokines. Since

the production of these cytokines is positively influenced by the number of times the cell has polliferated (Gett, 1998), the cell growth cycle was integrated into our simulation. With this comprehensive model we hope to understand how changes in the state of an individual cell would influence the environment and consequently may change the state of the collective. In addition, we hope to answer questions regarding the stability of the system to various perturbations, such as rewiring the GRN inside some/all cells, changing the parameters of the cell-to-cell interactions, and adding or killing cells.

2 Materials and methods

The various processes of the cell population are simulated as discrete events. Events are scheduled with an implementation of the Gillespie's stochastic simulation algorithm (Gillespie, 1976), that proceeds as follows.

1. Initialisation: Initialise the number of molecules in the system, reactions constants, and the random number generator.
2. Monte Carlo step: Generate random numbers to determine the next reaction to occur as well as the time interval (waiting time).
3. Update: Increase the time step by the randomly generated time in Step 2. Update the reactants based on the reaction that occurred.
4. Iterate: Go back to Step 2 unless the number of reactants is zero or the simulation time has been exceeded.

The Monte Carlo step is implemented by first randomly generating the waiting time for all the event (distributed as a negative exponential). Events are then sorted in ascending order of their waiting times. After an event has occurred, we regenerate the waiting time for those events in queue that were influenced by its occurrence. We further describe the various discrete events in our model and their associated parameters.

2.1 Activation of Th cells

The T helper cells develop in the thymus following which the matured, naive cells leave and circulate in the lymphoids. The naive cells are those which have not yet had contact with an APC they recognise. The newly developed Th cells begin to spread though the body via the lymphatic vessels that connect the lymph nodes. During an immune response, the antigen presenting cells absorb the foreign material, and travel from the infection site to the lymph nodes. Once at the lymph

nodes, the APC begins to present antigen peptides on its surface. This allows the Th cells that express the specific receptors (variable region of TCR) against the antigen to activate (Fig. 1).

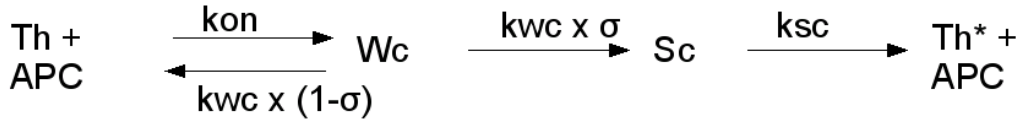

Figure 1: Flow diagram indicating interactions between Th cells and APCs. On contacting an APC with an available binding site, the Th cell proceeds to weakly conjugate with it (Wc). In case of APC with the same specificity, the Th cell then strongly conjugates (Sc). Parameters k_{on}, k_{wc}, k_{sc} and σ are described in Table 1.

Table 1: Description of model parameters of Th cells and conjugations with Antigen Presenting Cells (APC).

Parameters	Description	Value
k_{on}	Rate of contact between Th cells and APCs	3.6×10^{-10} $Liters.h^{-1}.cell^{-1}$
k_{wc}	Average time cell stays in weak conjugation	0.17 h
σ	Proportion of cognate conjugations	1 (for monoclonal antibodies)
k_{sc}	Average time cell stays strongly conjugated	1 h
τ_{cell}	Life span of Th cell	16 h

The Th cells bind to APCs with a mean waiting time of $1/(kon*nAPC)$, where $nAPC$ is the number of APCs in our simulation. Each APC has a predetermined number of binding sites to Th cells. When the "contact" event occurs, we select one of the APCs at random. In case the selected APC has a free binding site, we proceed with the weak conjugation of the Th cell to the APC at this site. However, if all the binding sites at the selected APC are occupied, we regenerate the Th cell - APC contact event.

2.2 Th cell differentiation pathways

The existing comprehensive logical model of regulatory networks and signalling pathways is utilised to simulate Th cell differentiation (Naldi et al., 2010). The 64 node network (Fig. 2) comprises the following.

1. Inputs to the network. The conjugation of a Th cell with an APC activates the corresponding network input. In addition, 12 input components indicate the presence of different cytokines in the environment.

2. Cytokine receptor chains on the surface of the cell.

3. Activator proteins (NFAT and STATs), activated by the receptors.

4. Cytokines produced by the cell namely IL-2, IL-4, IFNγ, IL-10, IL-21, IL-23 and TGFβ. These components can be thought of as outputs from the network.

5. The master transcription factors (Tbet, GATA3, RORγt, and Foxp3), that indicate the Th cell lineage.

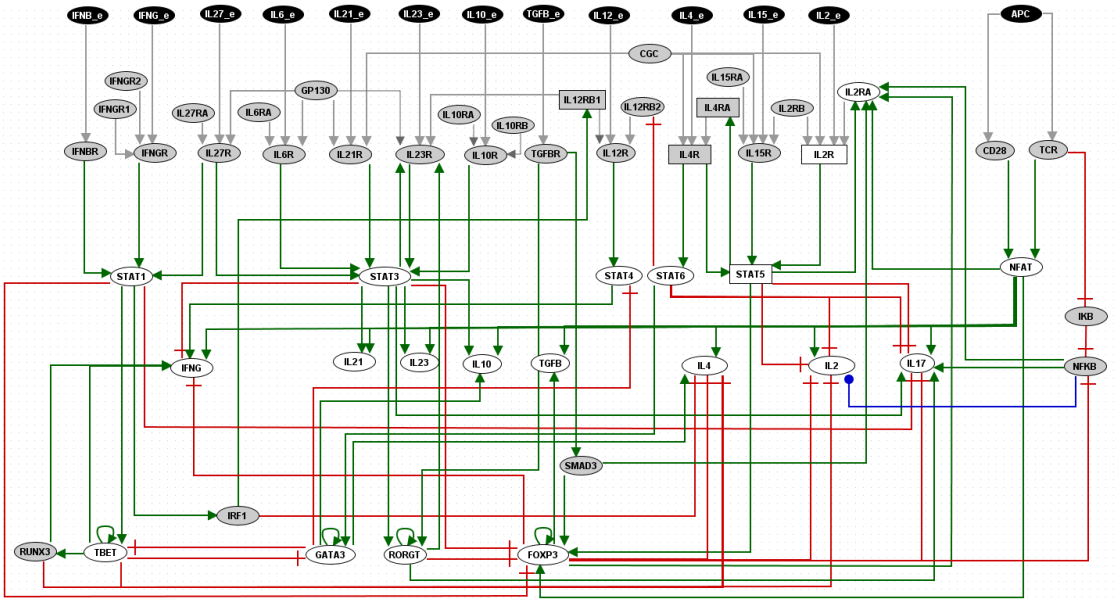

Figure 2: The Th cell differentiation regulatory graph, consisting of 64 components. Input components to the network and coloured black. Ellipses denote boolean components whereas rectangles denote ternary components (levels of activations at 0, 1 and 2). Activation and inhibitory connections are denoted by red and green arrows respectively. The blue arrow indicates a unique dual interaction, indicating the influence of NFKβ on IL-2 is dependent on the presence of other components.

Table 2: Average waiting times for three different classes of components of the cell logical network. The waiting times apply to both unitary increments and decrements of the component level of activation.

Component type	Average waiting time
Receptors	0.17 h
Activator proteins	1.5 h
Cytokines and master transcription factors	6 h

2.3 Cell growth cycle

The growth cycle of a cell consists of four major parts called phases. The first phase, termed G1 constitutes the synthesis of various enzymes required for DNA

replication. The cells enter this phase from the G0 or quiescent phase, after productive conjugation with an APC. The second phase labelled S, indicates the DNA synthesis resulting in two identical sets of chromosomes. This is followed by the G2 phase, wherein significant protein synthesis occurs (mainly involving the production of microtubules). The final M phase, consists of the actual nuclear and cytoplasmic division, accompanied by the formation of a new cell membrane. The Fig. 3 illustrates our model of this cycle.

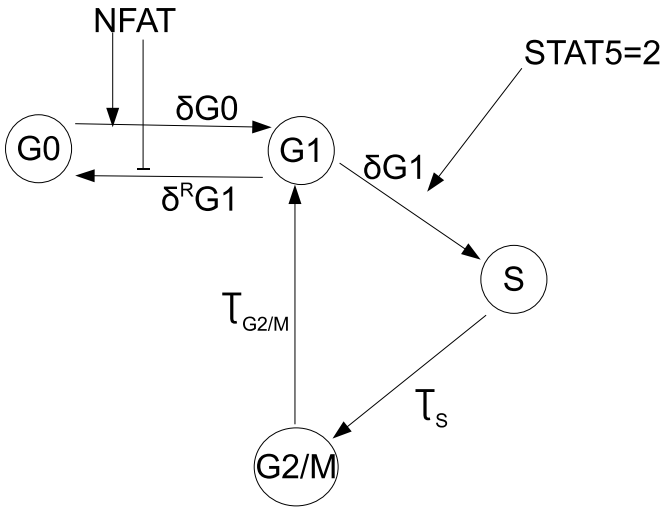

Figure 3: Cell growth cycle. The parameters are described in Table 3. The transitions between G0, G1 and S are influenced by the network components NFAT and STAT5 as indicated. Consequently the waiting times associated with these transitions are formalised as the time for NFAT or STAT5 to activate in addition to a parametrised residual time.

2.4 Environment

Cytokines act as the medium through with cells interact with each other. In our simulations, the cells produce and consume cytokines when the corresponding network components are activated. In addition, the cytokine inputs to the network are turned on when the corresponding cytokine concentration exceeds a pre-defined threshold. The modelled environment constitutes seven cytokines produced by the Th cells (IL-2, IL-4, IFNγ, IL-10, IL-21, IL-23 and TGFβ) and five cytokines produced by APCs (IFNβ, IL-6, IL-12, IL-15 and IL-27).

Table 3: Description of model parameters of Th cell growth cycle, parameter values from (Leon et al., 2004)

Parameters	Description	Value
σ_{G0}	Residual time in quiescent phase	46.5 h
σ_{G1}	Residual time in first growth phase	0.8 h
σ_{G1}^{R}	Residual time to revert to quiescent phase	24 h
τ_S	Average time to synthesise DNA	6 h
τ_{G2M}	Average time in second growth stage and mitosis	2 h

2.4.1 Cytokines produced by Th cells

The Th cell producing cytokines are assumed to be distributed uniformly throughout the medium. This allows us to simplify the computation of cytokine concentration (C) by integrating over the number of cells producing and consuming cytokines, irrespective of their positions in the medium. The amount of cytokine consumed by a Th cell is dependent on the amount of cytokine in the medium, modelled using a piece-wise linear equations Fig. 4.

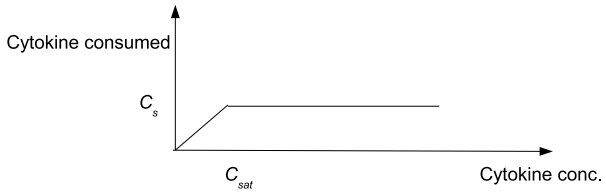

Figure 4: Caricature illustrating the consumption rate of cytokines by a single Th cell. Cs indicates the maximum consumption rate, and $Csat$ the cytokine concentration in the medium at which the consumption saturates

The maximum consumption rate Cs can be estimated using the following equation.

$$Cs = k_{in}RI \tag{1}$$

where RI is the number of cytokine receptors on the cell's surface (each receptor can bind to a cytokine molecule),
k_{in} is the rate of internalisation of cytokine molecules bound to the cell's surface.

In order to estimate the concentration at which the consumption saturates $Csat$, we consider quasi steady state conditions equating the rates at which cytokine molecules bind and unbind from the receptors at the cell's surface.

Consequently, the number of bound cytokine molecules can be expressed as a function of the amount of free cytokine in the medium, with the following equation.

$$C = \frac{C_B V}{k_a(RI - C_B)} \tag{2}$$

where C is the amount of free cytokine in the medium of volume V, C_B is the number of cytokine molecules bound to the cell surface and k_a is the affinity constant of this binding.

Cytokine concentration at which the consumption saturates $Csat$, can now be computed from Equation 2 as the amount of free cytokine (C) when all but one of the cytokine receptors are occupied.

The total cytokine concentration is computed with the following two equations.

$$C' = Sk_p - k_d - D \times C\frac{Cs}{Csat} \tag{3}$$

when $C < Csat$, otherwise

$$C' = Sk_p - k_d - D \times Cs \tag{4}$$

where
S: Number of cells producing cytokine at rate k_p
k_d: Degradation rate
D: Number of cells consuming cytokine
Cs: Maximum consumption rate and
$Csat$: Concentration at which the consumption saturates

The parameter values involved in cytokine production are listed in Tables 4 and 5.

2.4.2 Cytokines produced by Antigen Presenting Cells

On conjugation with an APC, the Th cell has access to the cytokines produced by that APC. Consequently, the Th cell's conjugation status along with threshold function maps the cytokine concentrations to network inputs. The cytokine concentration at an APC is computed as follows (parameters in Table 6).

$$C_{apc} = P - E_B Cs \tag{5}$$

Table 4: Description of model parameters for the paracrine cytokines IFNγ, IL-10, IL-21, IL-23 and TGFβ.

Parameters	Description	Value
k_p	Rate of of cytokine production by a Th cell	1000 $molec.h^{-1}$
RI	Number of cytokine receptors on cell	2000
k_{in}	Internalisation rate of cytokine	2.77 h^{-1}
C_s	Maximum rate of cytokine consumption by a Th cell	$RI \times k_{in} = 5540$ $molec.h^{-1}$
k_a	Affinity constant	0.001 pM^{-1}
C_{sat}	Cytokine concentration when consumption saturates	2 pM for supernatant volume of 1 μL
k_d	Degradation rate of cytokine	0.138 h^{-1}
θ	Threshold of response to cytokine concentration	0.2 pM (10% of receptors bound)

where P is constant amount of cytokine always present at the APC, E_B represents the number of Th cells conjugated to the APC, and Cs is the amount of cytokine consumed by each conjugated Th cell.

3 Results

Simulations were initially conducted on the logical network of a single cell. Corresponding to the work of Naldi *et al.* (2010), the network was initialised with the stable states corresponding to different Th cell lineages. Different environmental conditions known to stimulate Th cell differentiation into various lineages (Fig. 5), were then applied to the logical network of the cell and the corresponding stable states reached by the simulated network were recorded. For each of the initial network states and environmental conditions, simulations were repeated over 100 trials. Across all the trials, the cell lineages reached by the network were found to exactly match those established in Naldi et al. (2010) (Fig. 6).

Table 5: Description of model parameters for paracrine cytokines (IL-2 and IL-4) with ternary receptor components i.e., IL-2R, IL-4R and L4-RA.

Parameters	Description	Value
k_p	Rate of of cytokine production by a Th cell	1000 $molec.h^{-1}$
k_{in}	Internalisation rate of cytokine	2.77 h^{-1}
k_d	Degradation rate of cytokine	0.138 h^{-1}
RI_1	Number of cytokine receptors on cell when receptors at level of activation 1 (i.e. number of β and γ chains)	2000
C_{s1}	Maximum rate of cytokine consumption by a Th cell when $IL2R$ or $IL4R = 1$	$RI_1 \times k_{in} = 5540$ $molec.h^{-1}$
k_{a1}	Affinity constant when $IL2R$ or $IL4R = 1$	0.001 pM^{-1}
C_{sat1}	Cytokine concentration when consumption saturates for cells with $IL2R$ or $IL4R = 1$	2 pM for supernatant volume of 1 μL
RI_2	Number of cytokine receptors on cell when receptors at levels of activation 2 (i.e. number of α, β and γ chains)	2000
C_{s2}	Maximum rate of cytokine consumption by a Th cell when $IL2R$ or $IL4R = 2$	$RI_2 \times k_{in} = 5540$ $molec.h^{-1}$
k_{a2}	Affinity constant when $IL2R$ or $IL4R = 2$	0.1 pM^{-1}
C_{sat2}	Cytokine concentration when consumption saturates for cells with $IL2R$ or $IL4R = 2$	0.022 pM for supernatant volume of 1 μL
θ	Threshold of response to cytokine concentration	1 pM

4 Current investigations

We are currently simulating certain classical immunology experiments involving Th1 and Th2 cell population dynamics (Murphy et al., 1996; Gollob and Coffman,

Table 6: Description of model parameters for juxtacrine cytokines (IFNβ, IL-6, IL-12, IL-15, IL-27).

Parameters	Description	Value
P	Constant amount of cytokine present at APC	10 *molec*
C_s	Cytokine consumed by cell conjugated to APC	1 *molec*
θ	Threshold of response to cytokine concentration at the APC	0 *molec* (dependent on number of binding sites)

1994). These simulations would serve to set specific parameter values, mainly involving cytokine production and consumption by Th cells (Tables 4, 5 and 6). In addition, the results would allow us to verify specific mechanisms involved in the interactions between Th1 and Th2 cell populations.

Preliminary simulation results on the reversibility in differentiation of Th1 and Th2 cell types (Murphy et al., 1996) have indicated that primary stimulations of naive Th cells in the presence of the cytokine IL-12 result in the presence of a large proportion of IFN-γ producing cells, identified as Th1 cells. In contrast, primary stimulation of these naive cells with IL-4 and an anti IL-12 antibody results in IL-4 producing cells belonging to the Th2 lineage. We are now performing experiments to verify the extent to which differentiated Th1 cells can reverse into Th2 cells and vice versa.

5 Revisions to simulation

The profiling of the simulation code suggests that the sequential insertion of newly created events into the process queue while maintaining it sorted takes about 75% of the simulation time. We are currently implementing more optimal search algorithms for this purpose. The search time can be further reduced by inserting the events using a hierarchical data structure instead of the sequential process queue. This would allow us to sort only selected events in the queue, namely those that would fire first.

Another improvement we are currently integrating into the simulation, involves the use of a two compartment model to simulate the cytokine concentrations. The two compartments considered are 1. The cell vicinity pool and, 2. Remaining

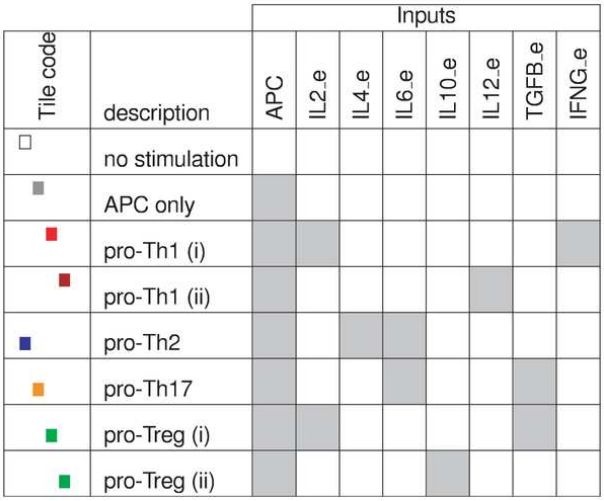

Figure 5: Environmental conditions used to stimulate the logical network. Each row represents a single prototypic environment. Grey and white cells indicate respectively the presence and absence of different inputs. The coloured tile code is used to represent the environmental conditions in the state transition table (Fig. 6) (Naldi et al., 2010).

pool. The vicinity pool constitutes the cells producing the cytokine (APCs in case of juxtacrine cytokines, and Th cells for paracrine cytokines) as well as cells in the immediate neighbourhood that consume this cytokine. Consumer cells outside of this neighbourhood are considered part of the remaining pool. With such an approach, one could model the diffusion of cytokines between the two pools. In addition, the different consumption rates for cells in the two pools would serve to be more accurate and we would be better able to estimate the parameter values involving juxtacrine cytokine concentrations.

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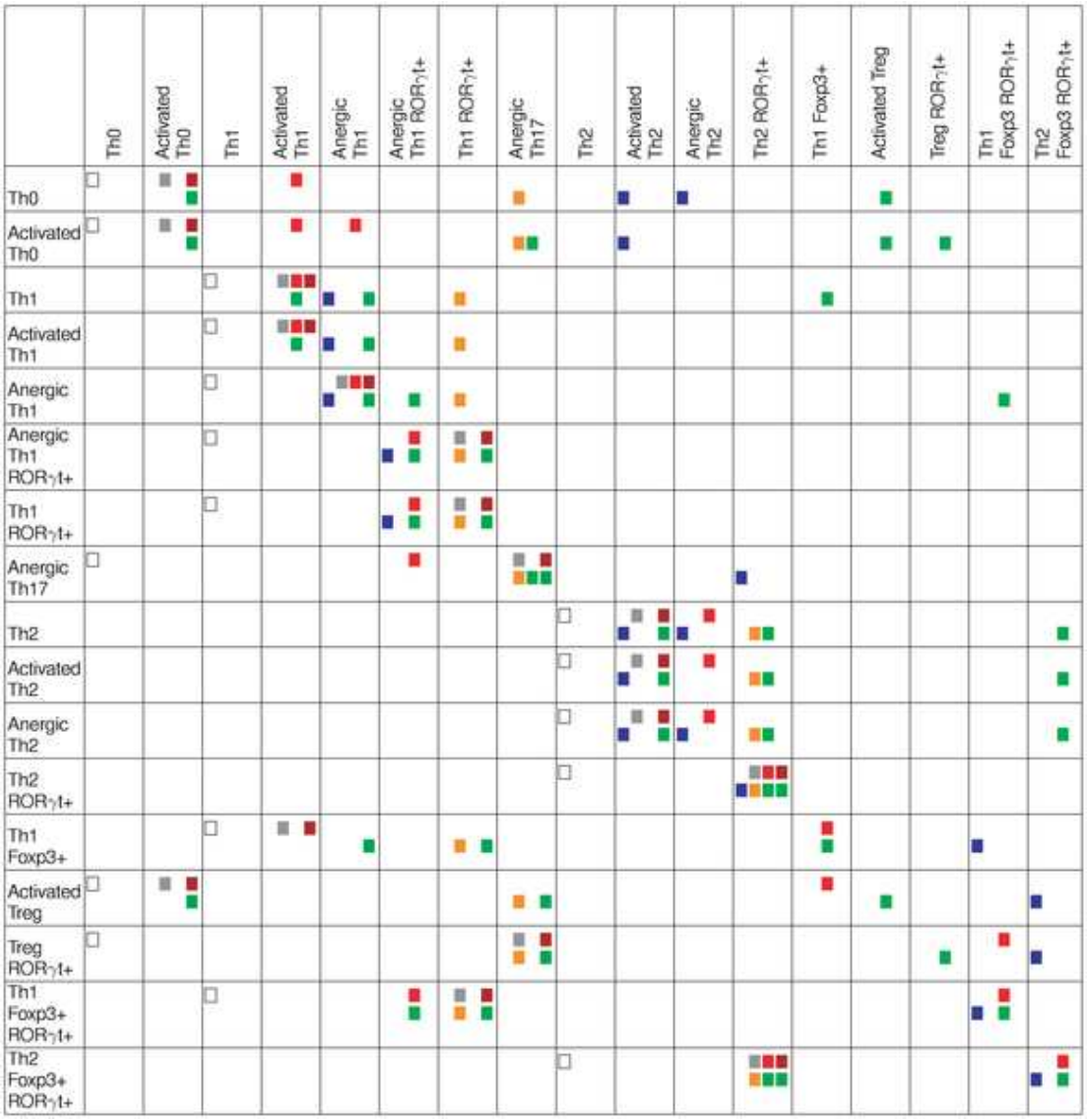

Figure 6: Context-dependent stable states (column entries) reached by the logical network, depending on initial states (row entries) and environmental conditions (coloured tiles). The coloured tile code for environmental conditions is defined in Fig. 5 (Naldi et al., 2010).

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