Logical modelling of cell fate specification

Denis Thieffry

Contents

- Logical modelling of regulatory networks
- Application to T-helper cell differentiation
- Conclusions and prospects

4th COMBINE meeting
Paris, September 16th, 2013

Logical variations: Model definition

Boolean equations	Use of Boolean operators NOT, AND, and OR	Kauffman
Regulatory graph	Boolean rules derived directly from the graph (e.g. sums of positive/negative inputs compared to thresholds)	Borhnoldt Li
Bipartite graph	Introduction of AND nodes (regulations converging onto a components are combined with OR)	Klamt, Saez-Rodriguez
Regulatory graph + Boolean functions	The regulatory graph constraints the definition of the logical function	Demongeot Goles Irons
Regulatory graph + multilevel functions	The regulatory graph constraints the definition of the logical function	Thomas Snoussi
Fuzzy logic	A third symbolic value enables logical computation with unknown levels	Lauffenburger Sorger

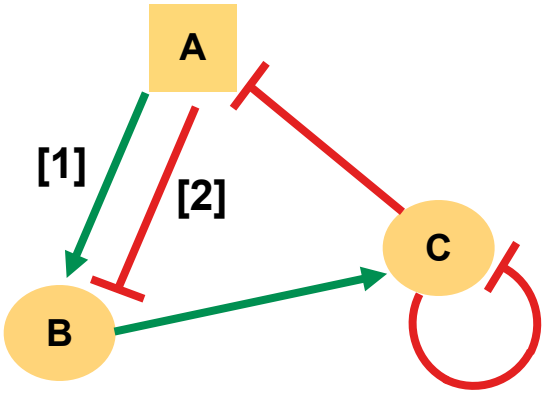
Logical variations: updating schemes

Synchronous	All components are updated simultaneously (deterministic paths)	Kauffman
Semi-synchronous	Use of dummy nodes to delay defined components (deterministic paths)	Albert, Chaves, Irons
Bloc synchronous	Components from a same bloc are updated synchronously following rank (deterministic paths)	Goles, Demongeot
Fully asynchronous	All enabled single transitions are considered (non deterministic transition graph)	Thomas
Time delays	Association of continuous clocks with components	Thomas, Bockmayr
Complete	Consider all enabled a/synchronous transitions	???
Mixed	Synchronous or asynchronous priority classes (non deterministic transition graph)	Fauré et al (2006, 2009)

Assets of logical modelling

- Exploitation of heterogenous, incomplete and/or qualitative data
- Versatility (e.g. consideration of different levels of abstraction)
- Bottom up approach (easy composition)
- Rigorous formal framework
- Scaling up potential, e.g. taking advantage of reduction methods
- Straightforward simulation of perturbations (KO, KI, etc.) => predictive power
- Powerful simulation and analysis tools
- SBML Qual exchange format - http://arxiv.org/abs/1309.1910

Logical modelling of regulatory networks



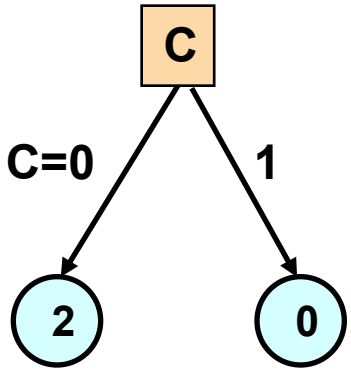
- ✓ A **graph** describes the interactions between genes or regulatory products
- ✓ **Discrete levels** of expression associated to each regulatory component and interaction

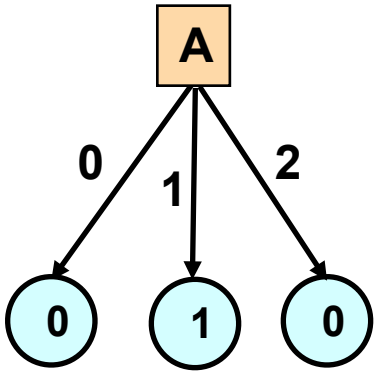
Logical rules/parameters

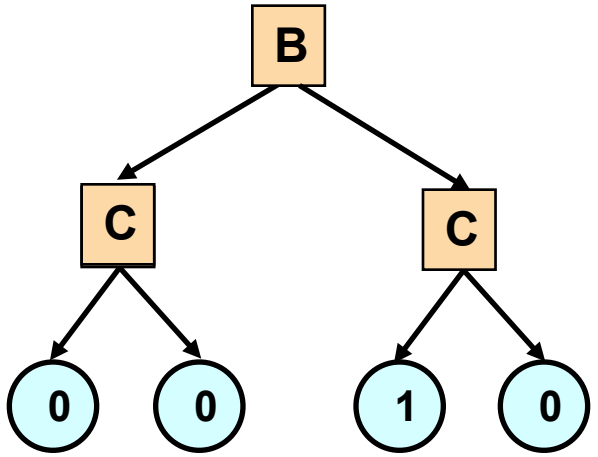
K_A = 2 IFF (C=0)
K_A = 0 otherwise

K_B = 1 IFF (A=1)
K_B = 0 otherwise

K_C = 1 IFF (B=1) AND (C=0)
K_C = 0 otherwise

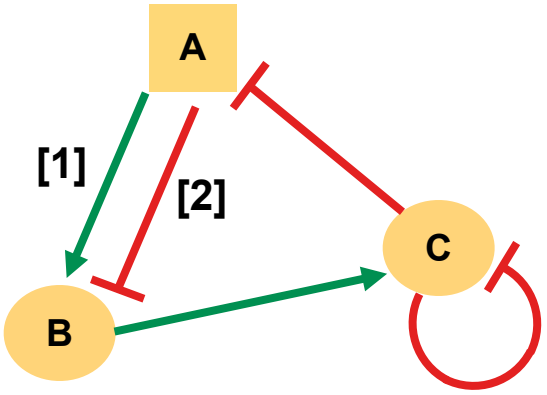






Decision trees

Logical modelling of regulatory networks



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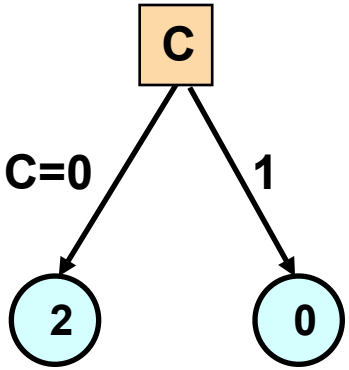
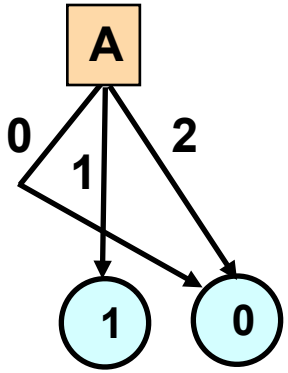
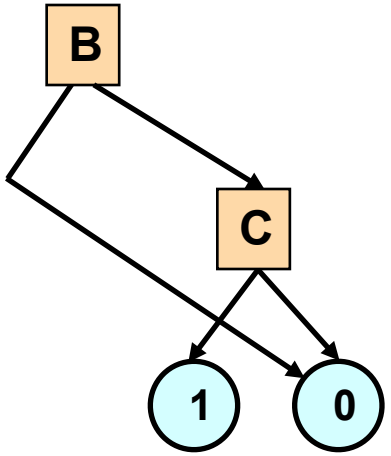
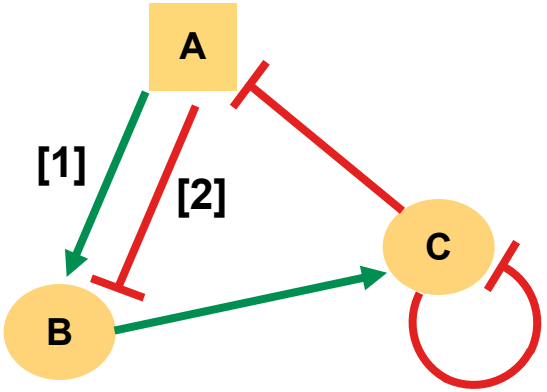






Decision diagrams

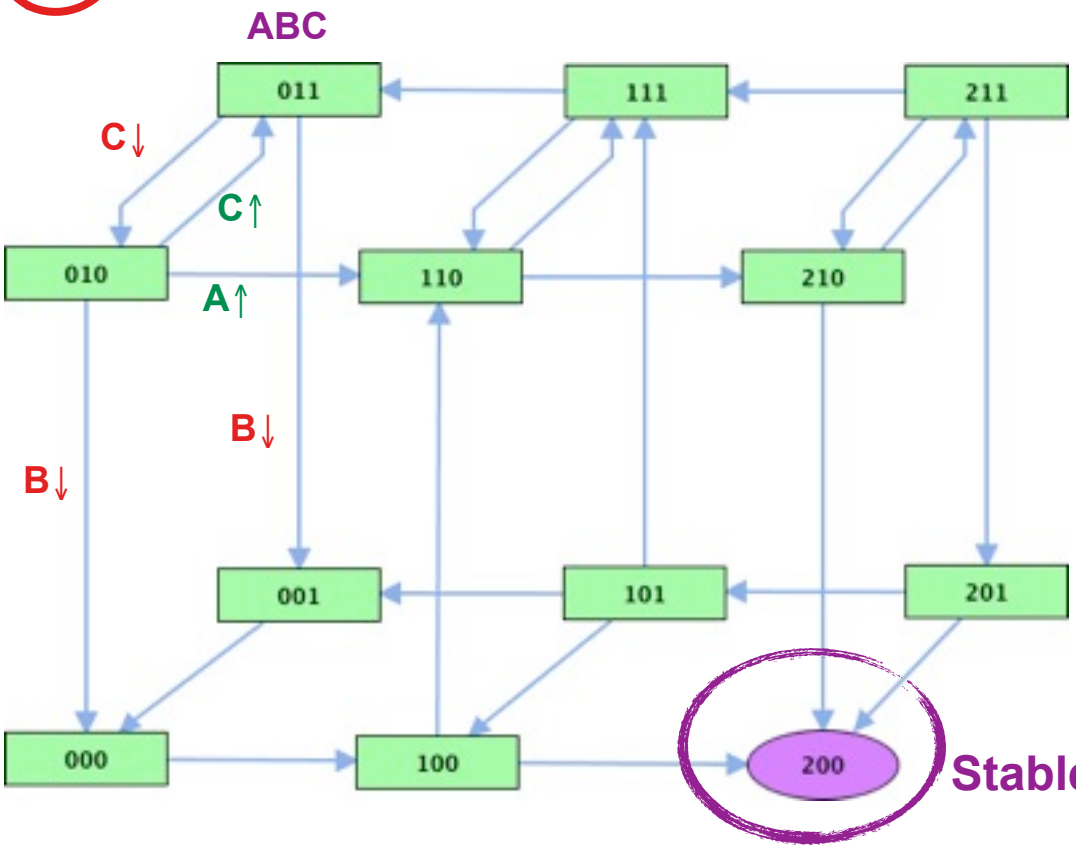
Logical state transition graphs



Regulatory graph + Logical rules
=> simulations / dynamical analysis

Asynchronous updating (R Thomas)

State transition graph



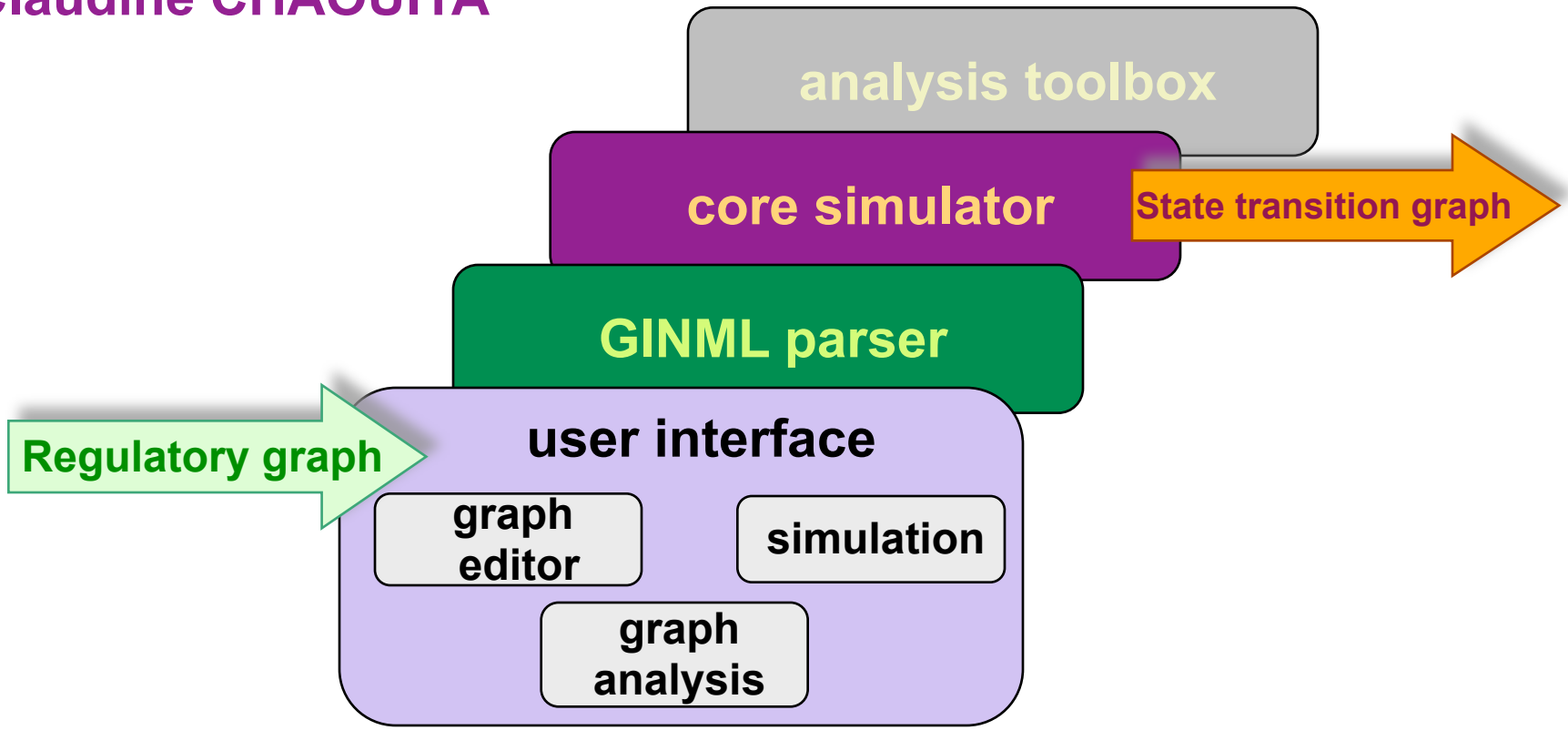
Development of dynamical analysis tools

- **Decision diagrams**
  - Identification of attractors
  - Analysis of regulatory circuits
  - Model reduction
  - State transition graph compression
- **Priority classes**
  - Mixed a/synchronous simulations
- **Petri nets**
  - Standard Petri nets
  - Coloured Petri nets
- **Model checking**
  - Verification of dynamical properties (temporal logic)
- **Logical programming**
  - Attractor identification and reachability analysis

GINsim (Gene Interaction Networks simulation)

Aurélien NALDI
Pedro MONTEIRO
Claudine CHAOUIYA

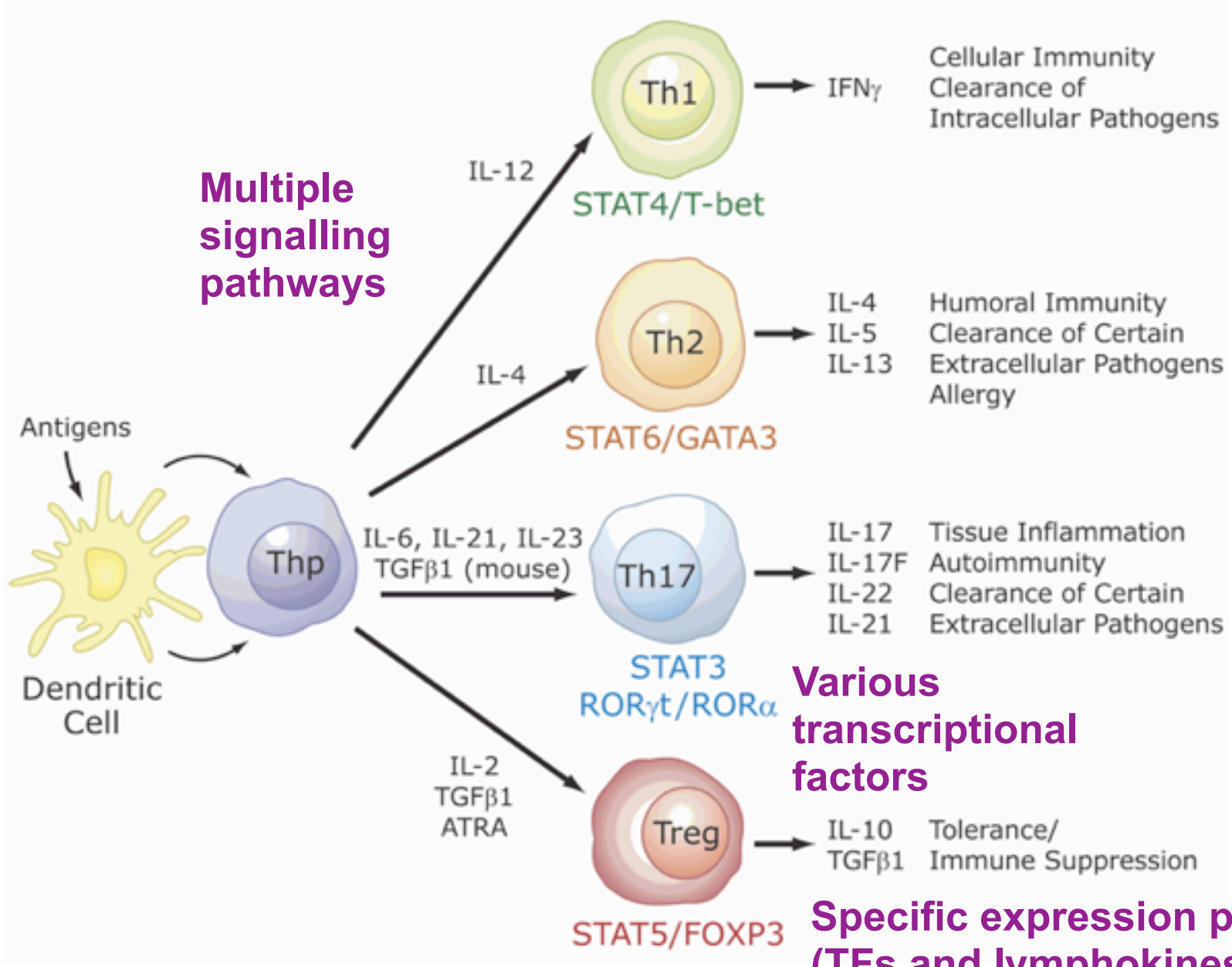
Duncan BERENGUIER
Lionel SPINELLI



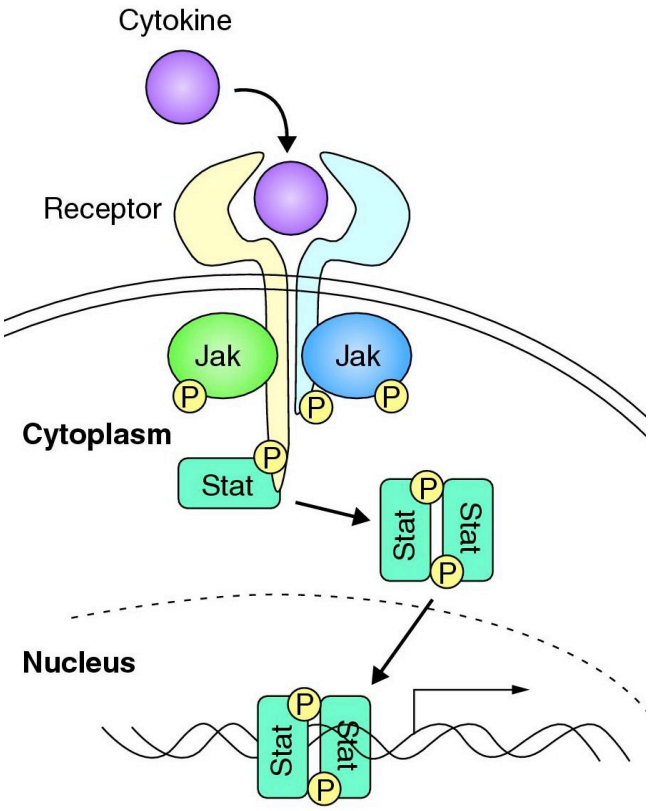
Available at
http://ginsim.org

Chaouiya *et al* (2012)
Meth Mol Biol **804**: 463-79

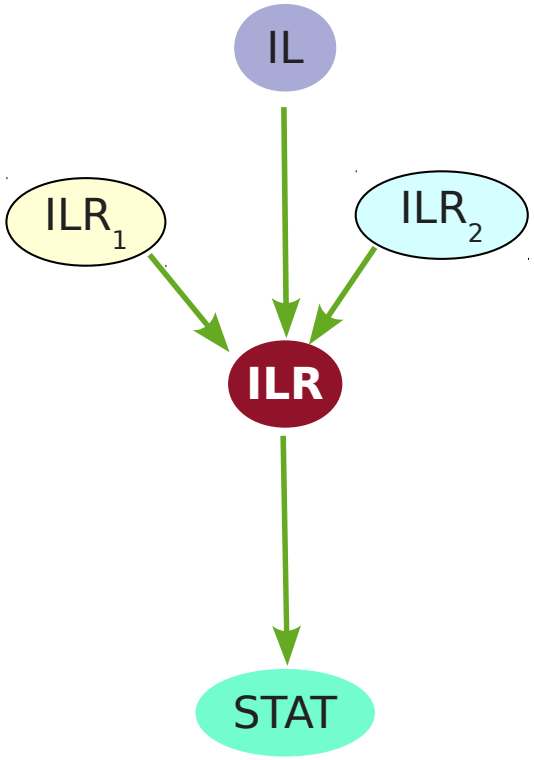
CD4+ T-helper cell differentiation



Towards a comprehensive, modular logical model of the Th differentiation network

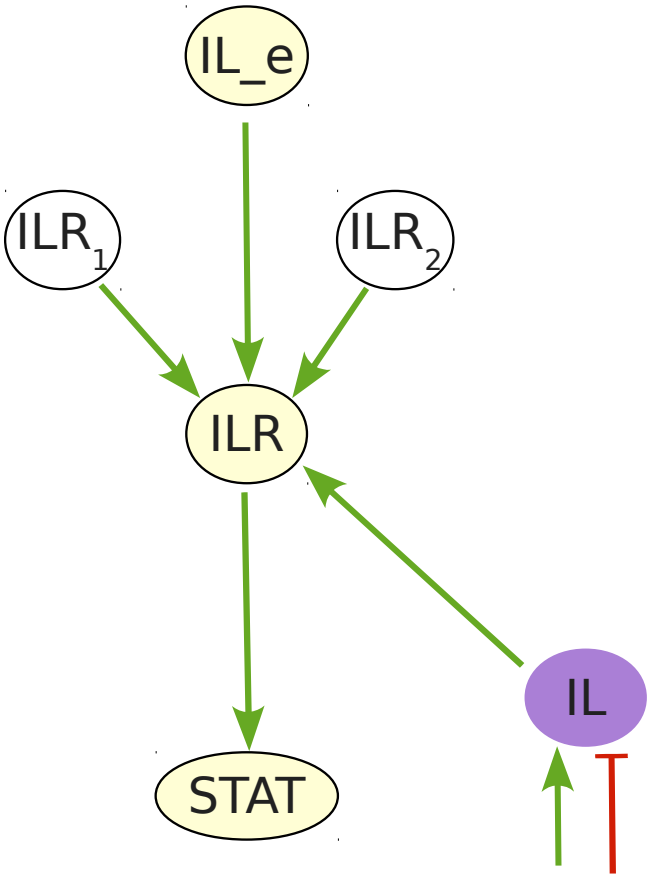


Yamoka *et al* (2004)



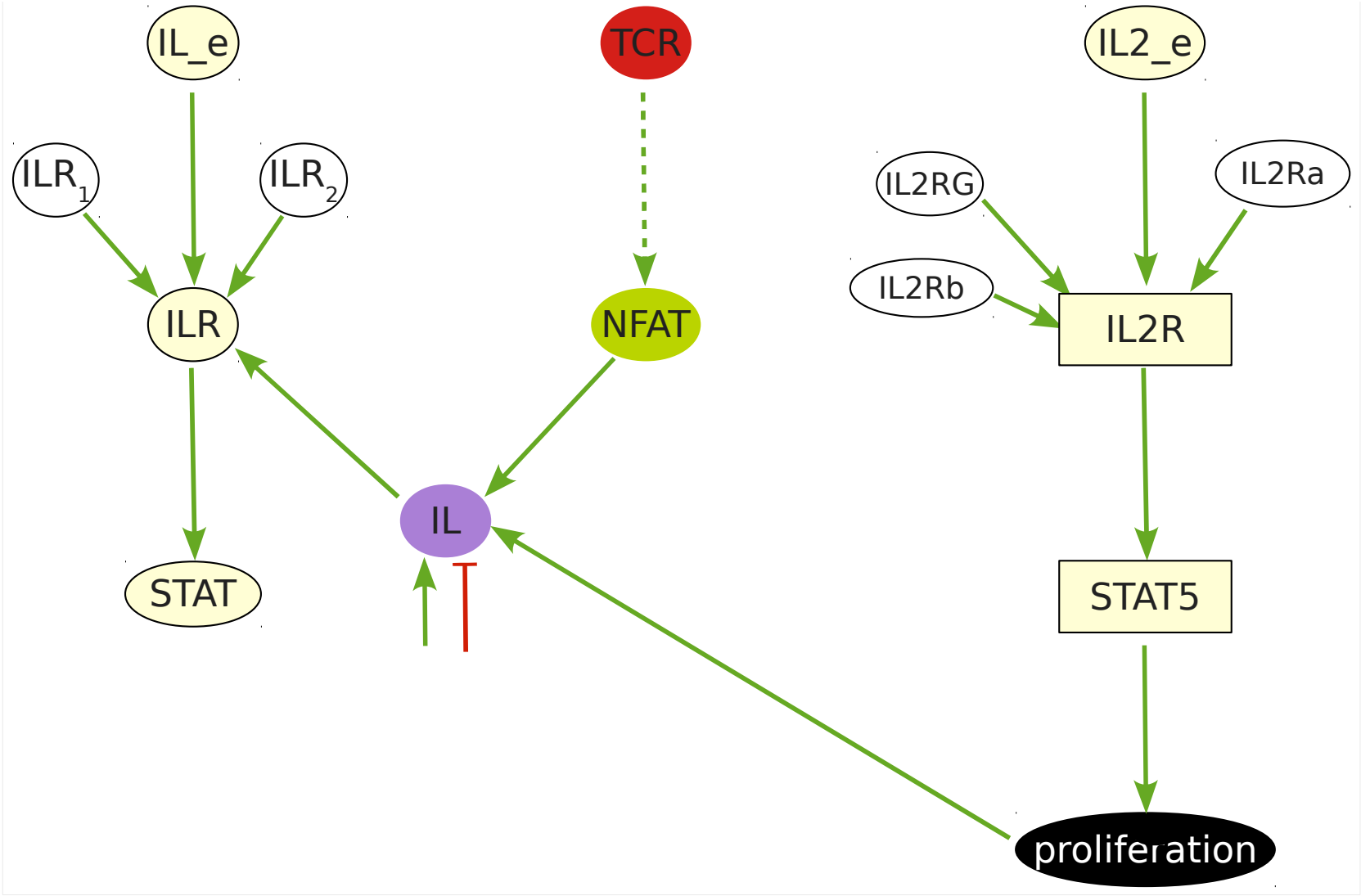
ILR = 1 **IFF** *IL* **AND** *ILR1* **AND** *ILR2*

Logical modelling of the Th network

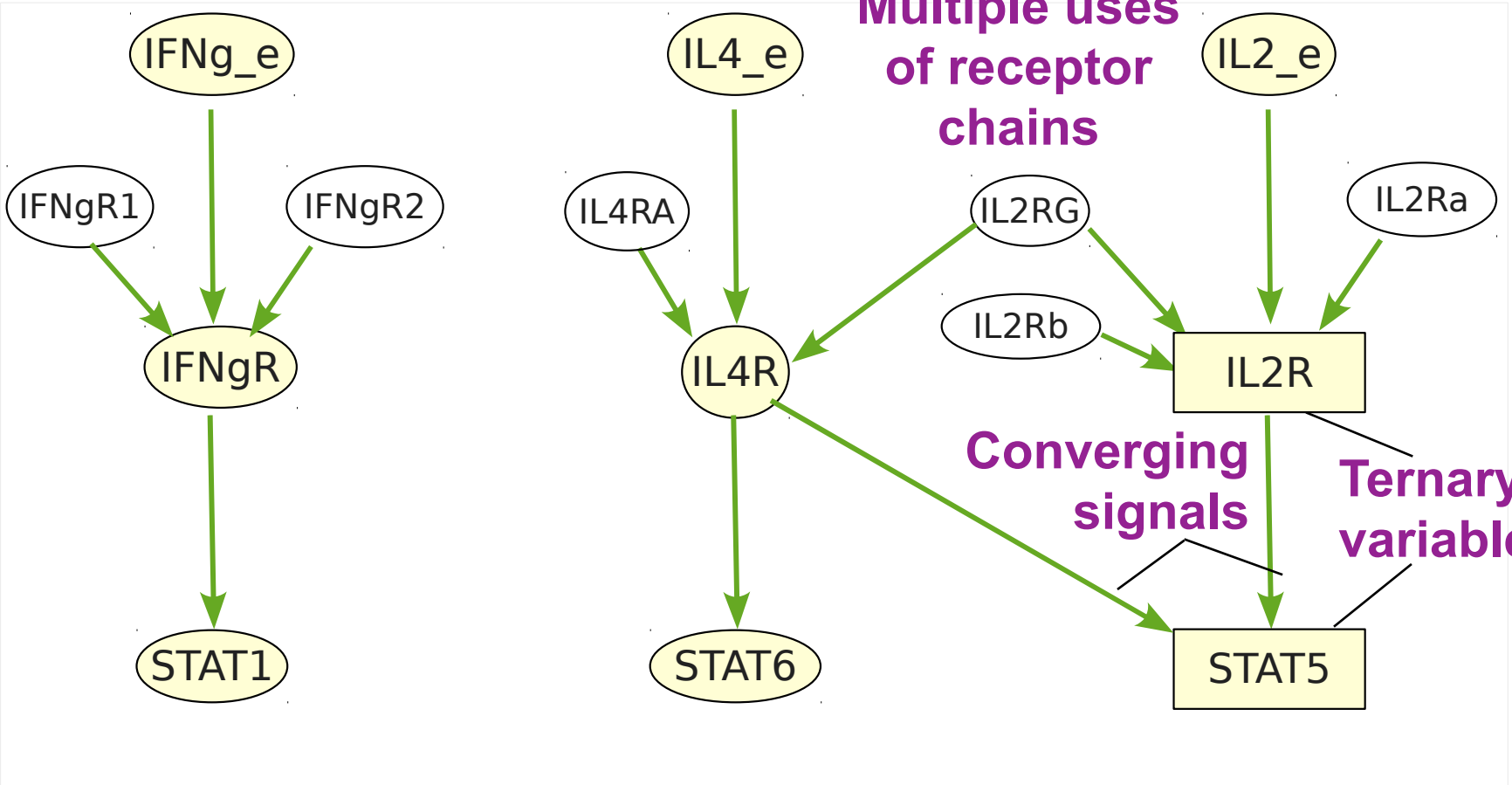


ILR = 1 **IFF** (*IL* **OR** *IL_e*) **AND** *ILR1* **AND** *ILR2*

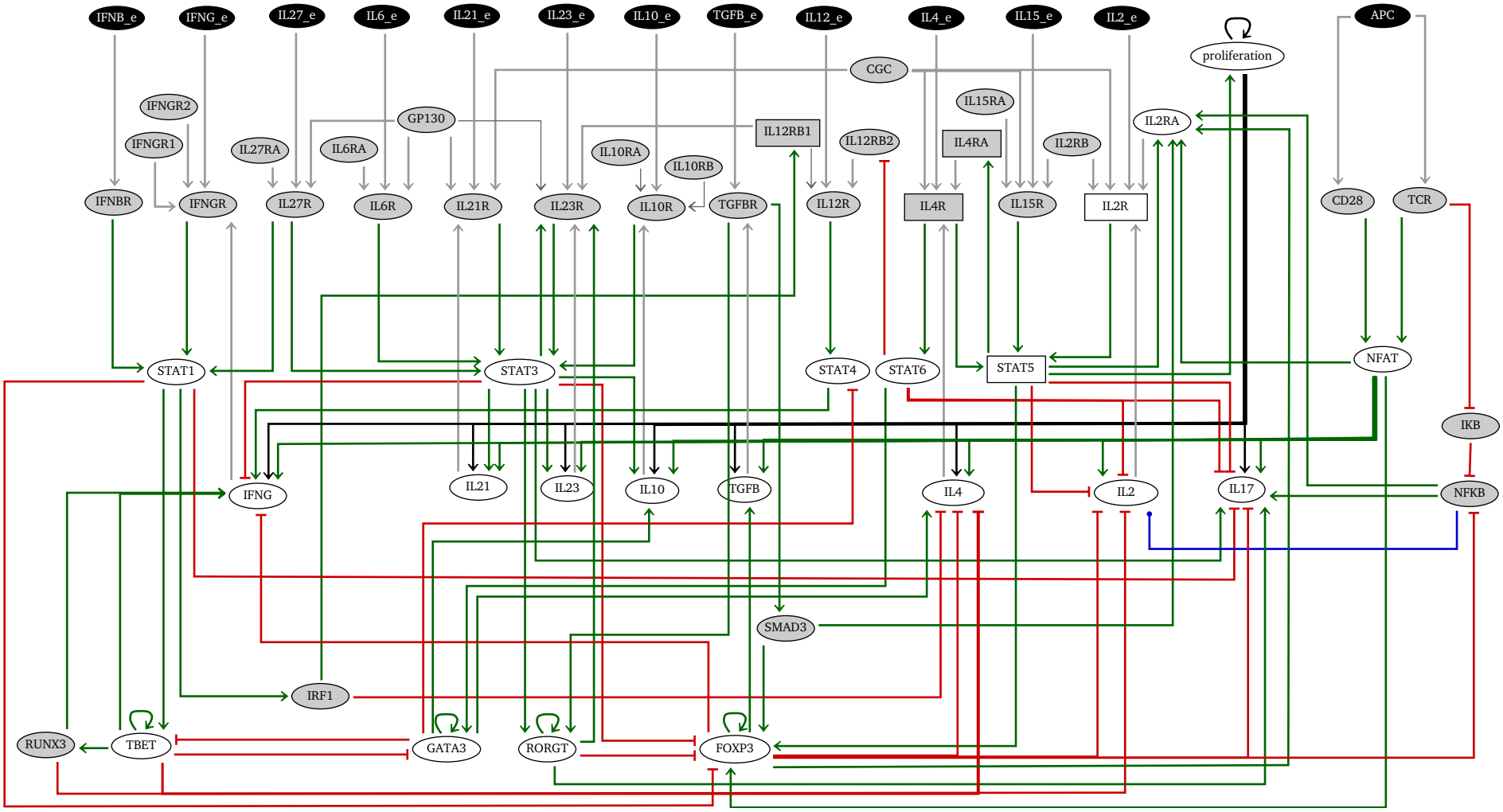
Logical modelling of the Th network



Logical modelling of the Th network



Logical model for the Th network

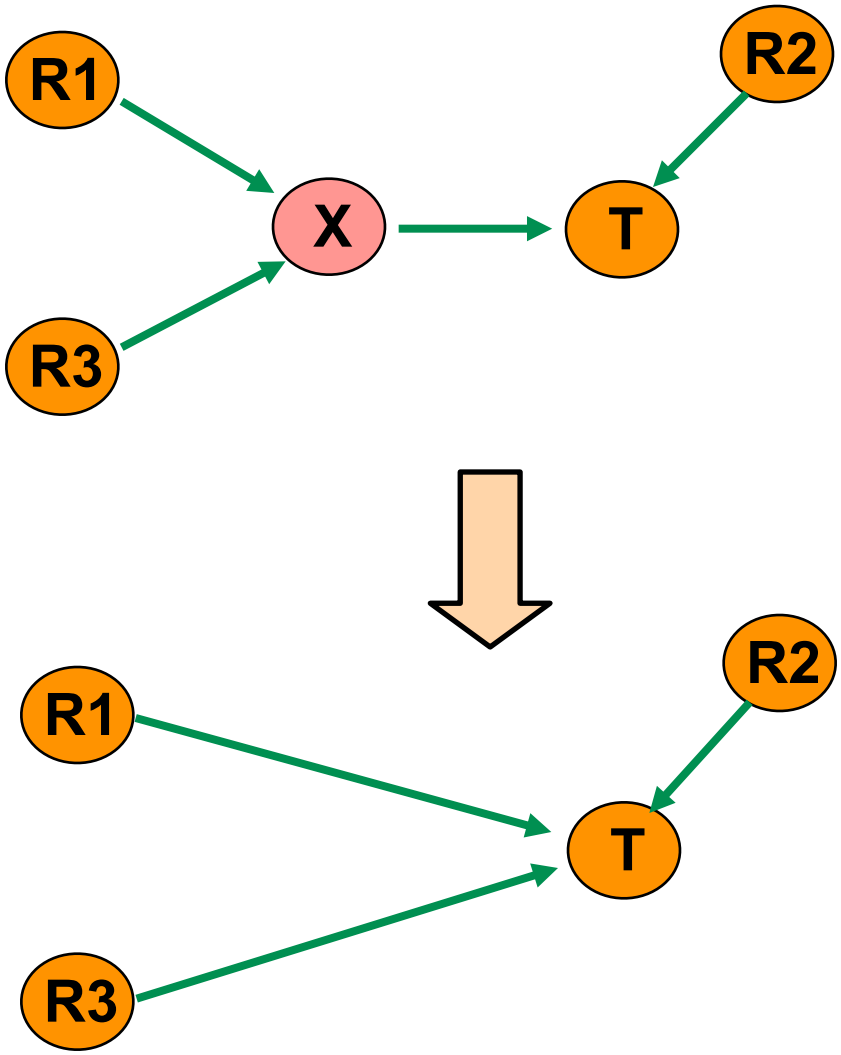


13 input components, 52 internal components, 339 circuits

=> too large to perform simulations

Naldi *et al* (2010)
PLoS Comput Biol ***6***: e1000912

Model reductions

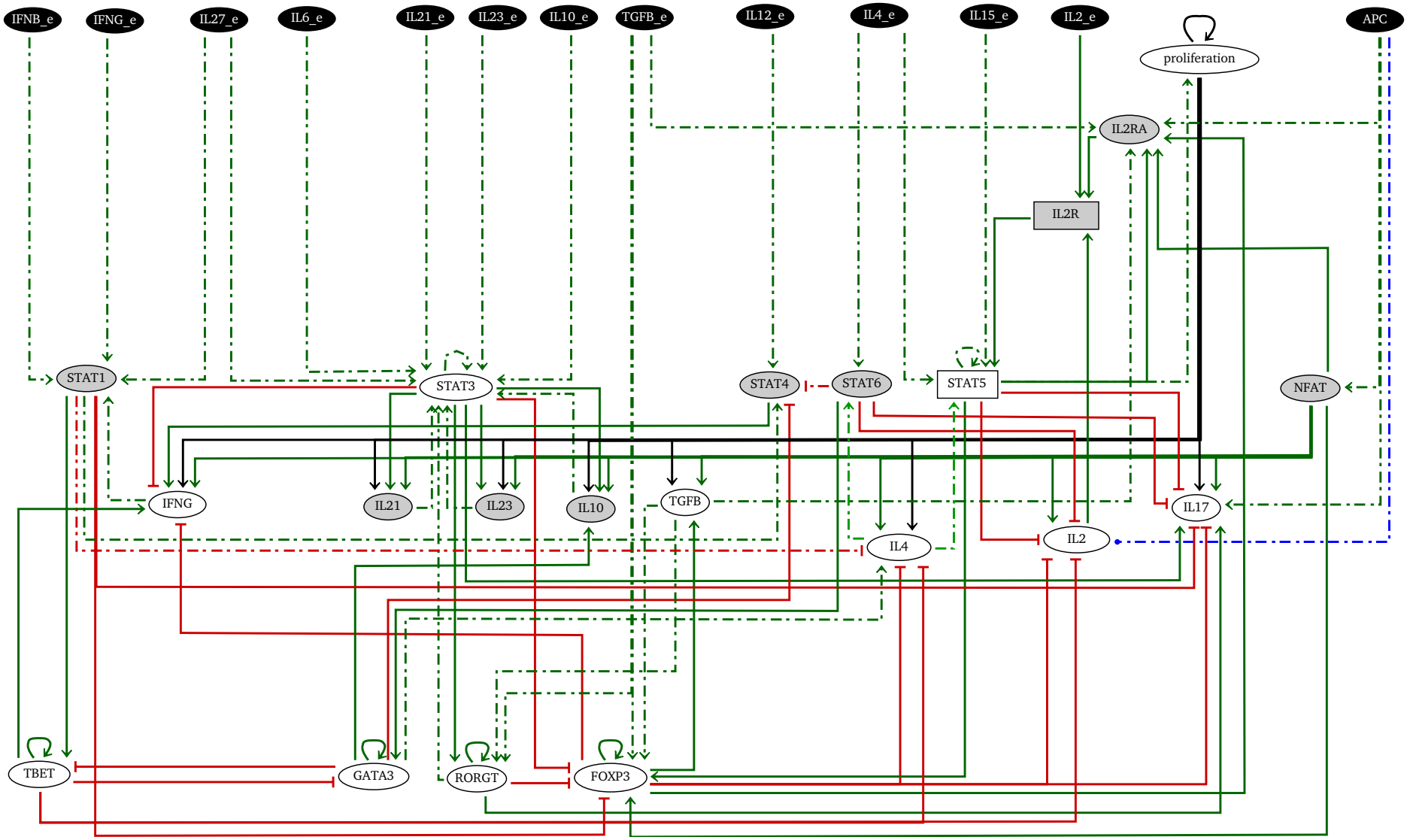


- **Keep the detailed model**
- **Reduction before analysis**

  => New rules for targets of hidden nodes
- **Choice of reduction**
- **Dynamical consistency**
  - **No** circuit deletion
  - Same stable states
  - Reachability may change

Naldi *et al* (2011)
Theor Comput Sci **412**: 2207-18

Reduced logical model

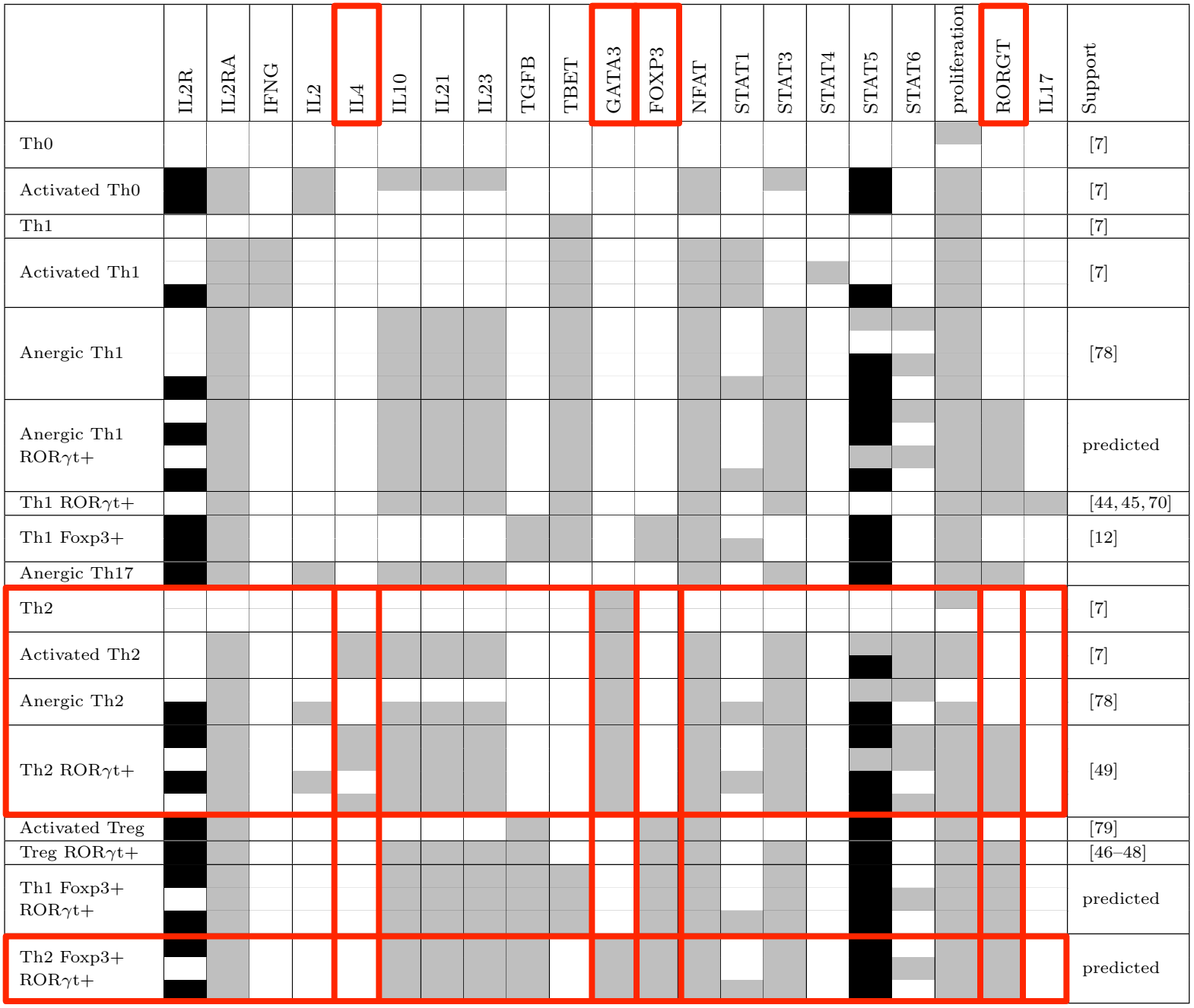


13 input components, 21 internal components

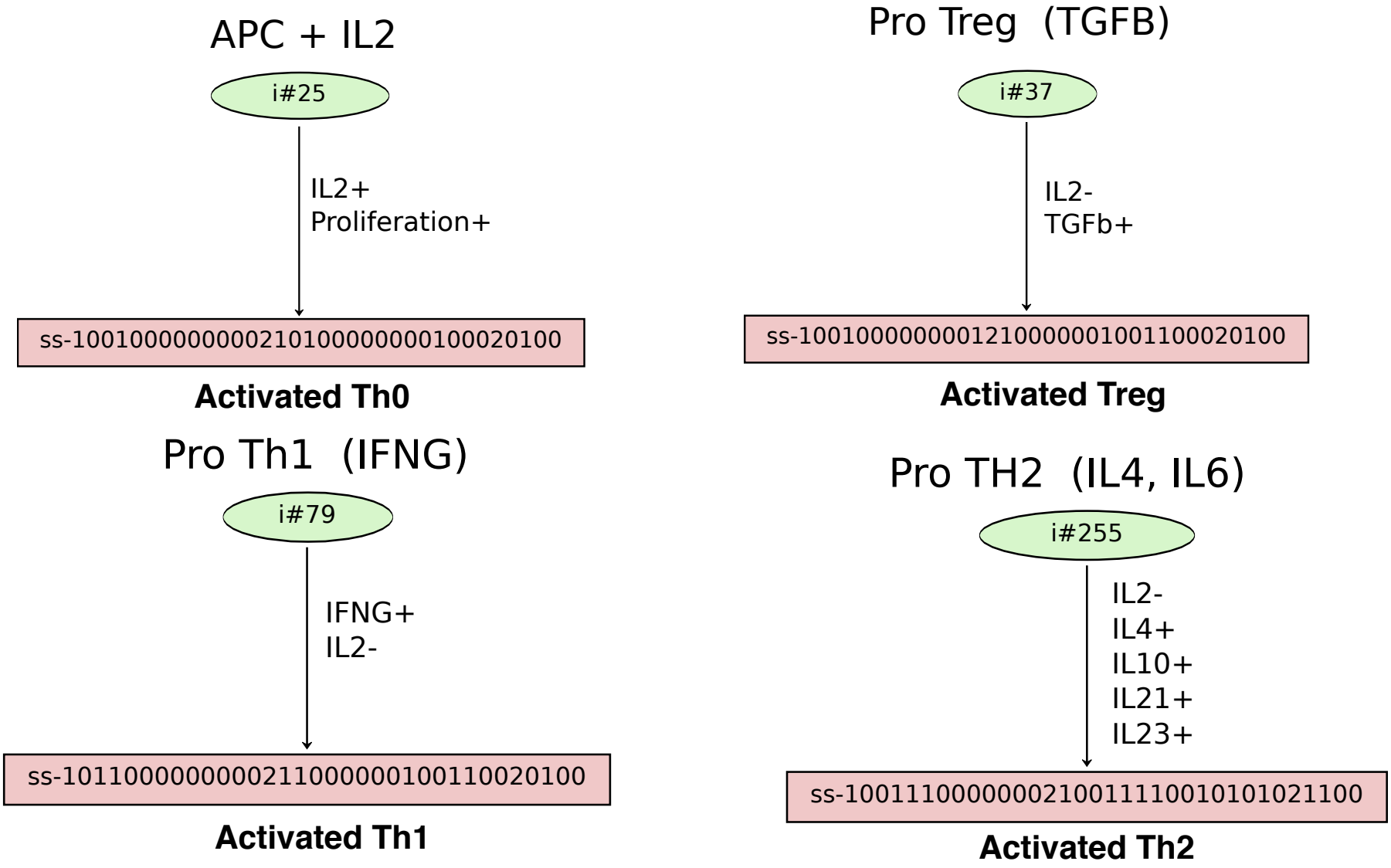
Selected environments for simulations

	APC	IL2	IL4	IL6	IL10	IL12	IFNG	TGFB
No input								
APC								
Pro-Th1								
Pro-Th1’								
Pro-Th2								
Pro-Th17								
Pro-Treg								
Pro-Treg’								

Stable signatures



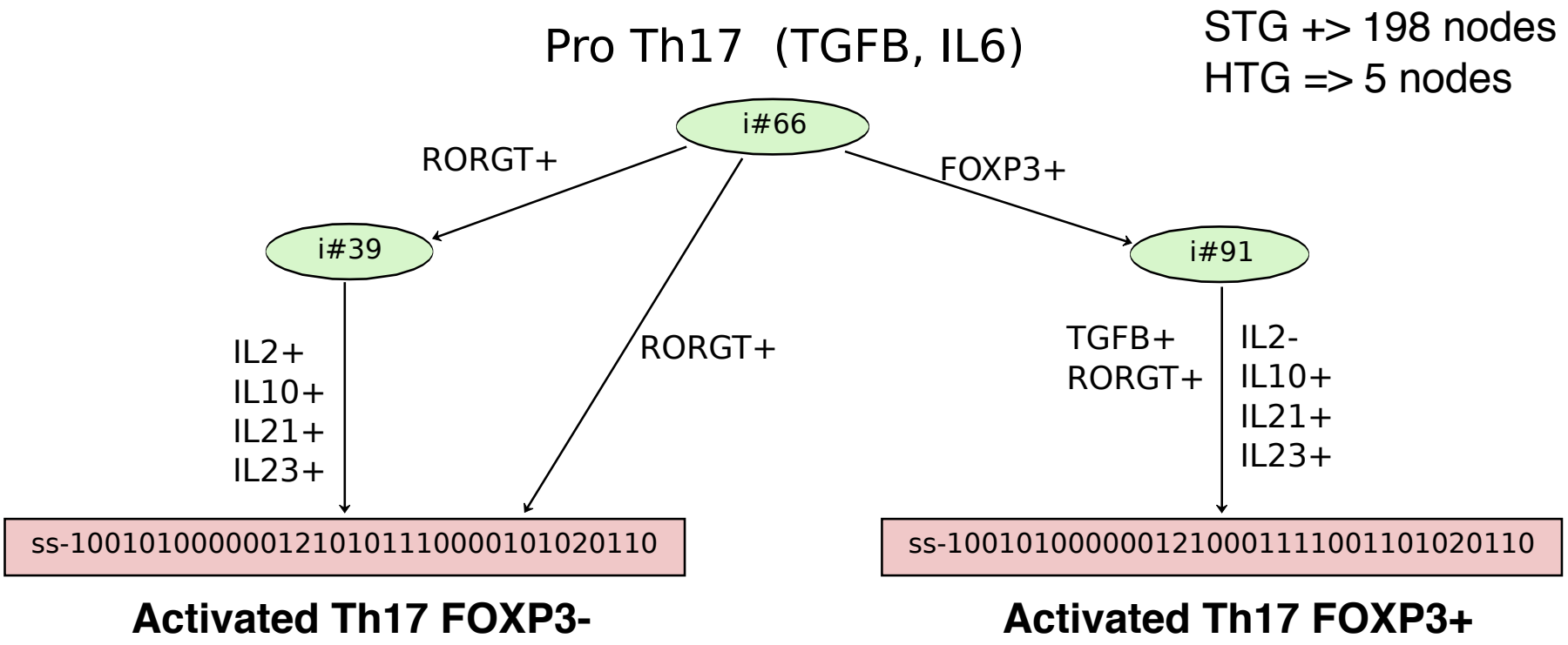
Simulations (Hierarchical Transition Graphs)



Node order: APC, IFNB_e, IFNG_e, IL2_e, IL4_e, IL6_e, IL10_e, IL12_e, IL15_e, IL21_e, IL23_e, IL27_e, TGFB_e, IL2R, IL2RA, IFNG, IL2, IL4, IL10, IL21, IL23, TGFB, TBET, GATA3, FOXP3, NFAT, STAT1, STAT3, STAT4, STAT5, STAT6, Proliferation RORGT and IL17.

Bérenguier *et al* (2013) *Chaos* ***23****: 025114*

Simulations (HTG)



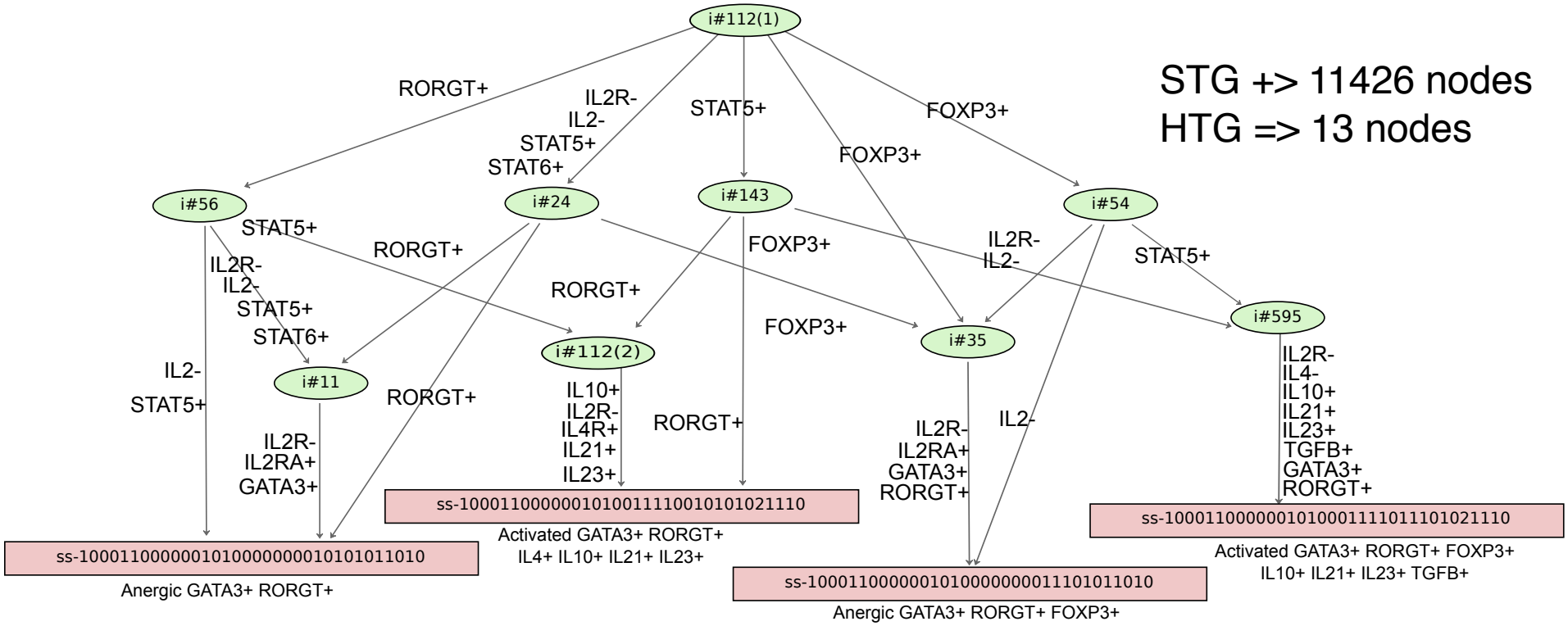
Node order: APC, IFNB_e, IFNG_e, IL2_e, IL4_e, IL6_e, IL10_e, IL12_e, IL15_e, IL21_e, IL23_e, IL27_e, TGFB_e, IL2R, IL2RA, IFNG, IL2, IL4, IL10, IL21, IL23, TGFB, TBET, GATA3, FOXP3, NFAT, STAT1, STAT3, STAT4, STAT5, STAT6, Proliferation RORGT and IL17.

Bérenguier *et al* (2013) *Chaos* ***23****: 025114*

Simulations (HTG)

APC + IL4 + IL6 + TGFB

(pro Th2 + Th17 cytokines, in the absence of IL2)



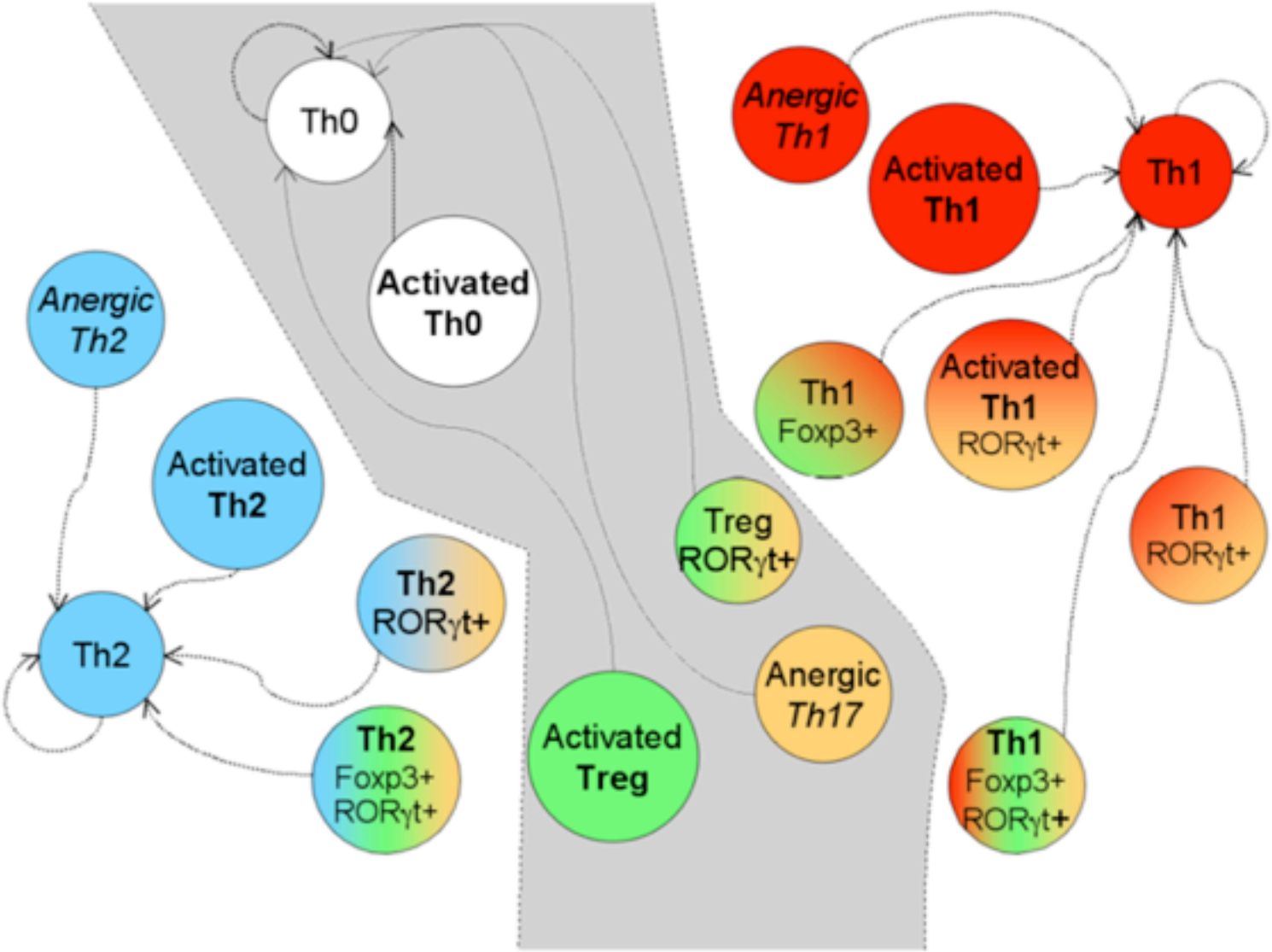
STG +> 11426 nodes

HTG => 13 nodes

Node order: APC, IFNB_e, IFNG_e, IL2_e, IL4_e, IL6_e, IL10_e, IL12_e, IL15_e, IL21_e, IL23_e, IL27_e, TGFB_e, IL2R, IL2RA, IFNG, IL2, IL4, IL10, IL21, IL23, TGFB, TBET, GATA3, FOXP3, NFAT, STAT1, STAT3, STAT4, STAT5, STAT6, Proliferation RORGT and IL17.

Bérenguier *et al* (2013) *Chaos* ***23****: 025114*

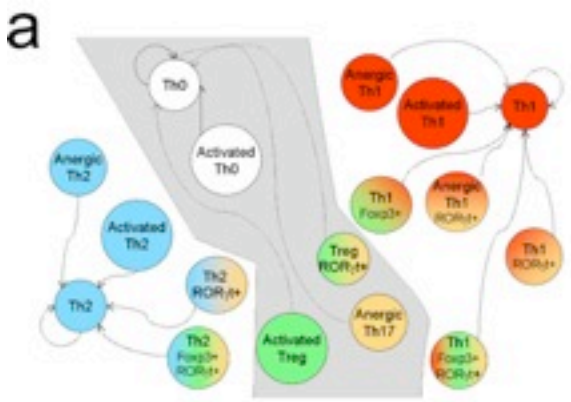
Simulations in the absence of stimulation



GATA3, Tbet, Foxp3 and RORγt

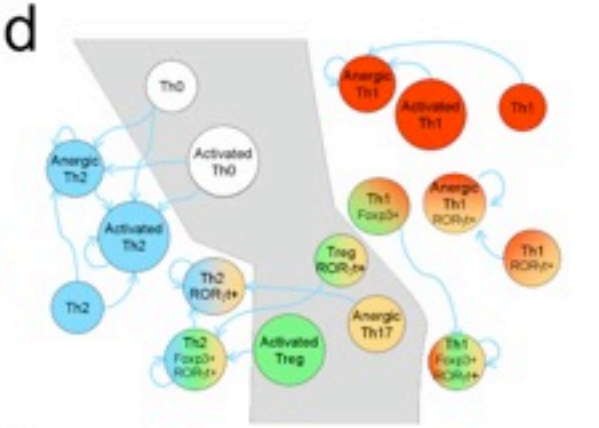
Overview of the simulation results for ≠ micro-environments

Absence of stimulation



APC only

Pro-Th1
IL2 & IFNg
or IL12



Pro-Th2
IL4 & IL6

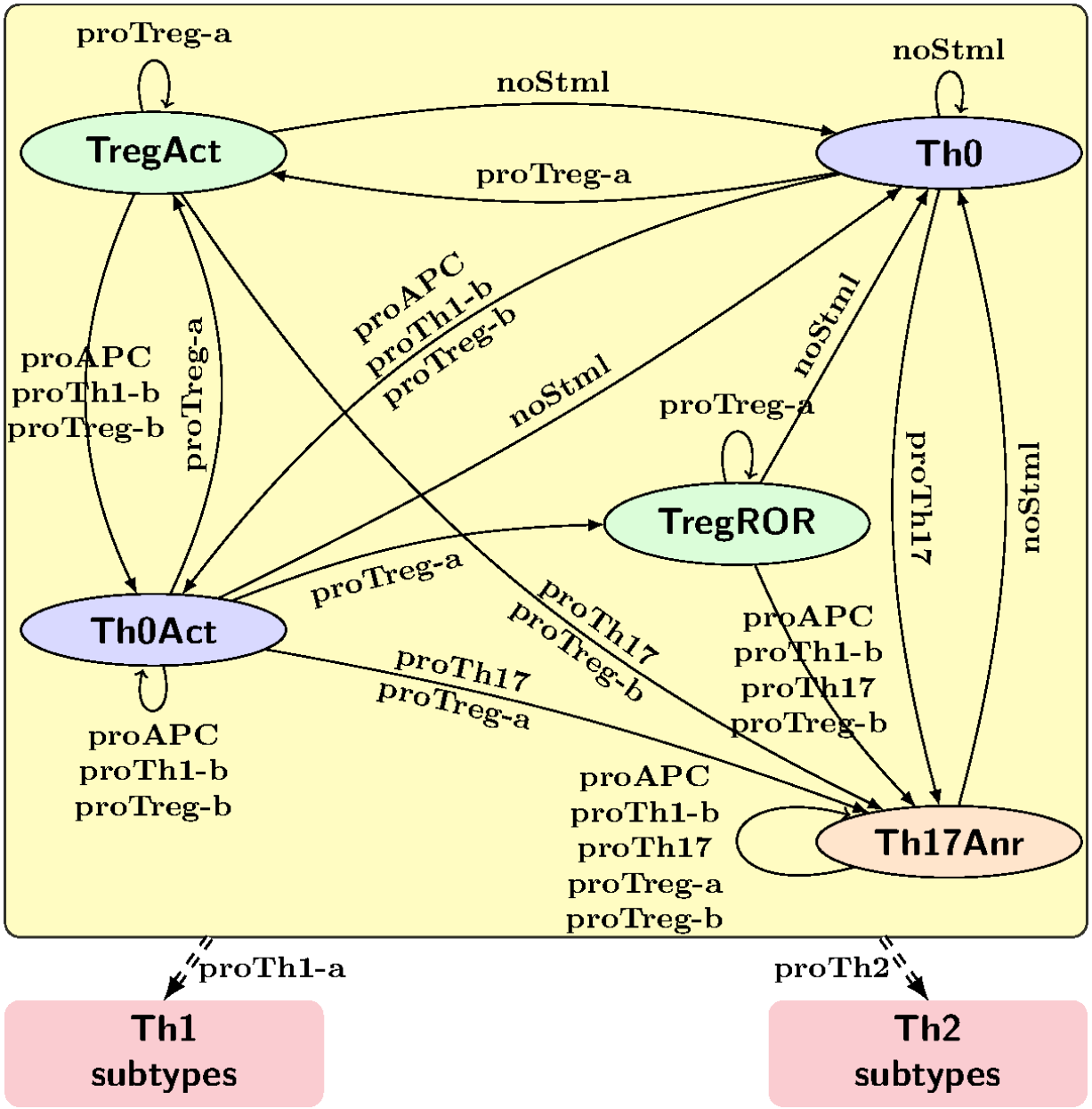
Pro-Treg
IL2 & TGFb
or IL10

Pro-Th17
IL6 & TGFb

GATA3 **Tbet**
Foxp3 **RORγt**

Naldi *et al* (2010)
PLoS Comput Biol ***6***: e1000912

Use of model checking to explore cell plasticity



- Export of the model from GINsim into **NuSMV** format
- Checking of the stability of Th **signatures** + **conversions** for changing inputs
- **Graph** displaying cellular conversion for specific input configurations (arc labels)

Bérenguier *et al* (2013)
Chaos ***23****: 025114*

Conclusions

- **Model** reproducing the main reported **Th subtypes** (Th0, Th1, Th2, Treg, Th17) in terms of stable states
- Many more stable states depending on signalling environment, including **hybrid subtypes**
- **Differentiation network** rather than **lineage tree**
- **Plasticity** of Th subtypes depending on signalling environment

Mechanisms Underlying Lineage Commitment and Plasticity of Helper CD4$^+$ T Cells

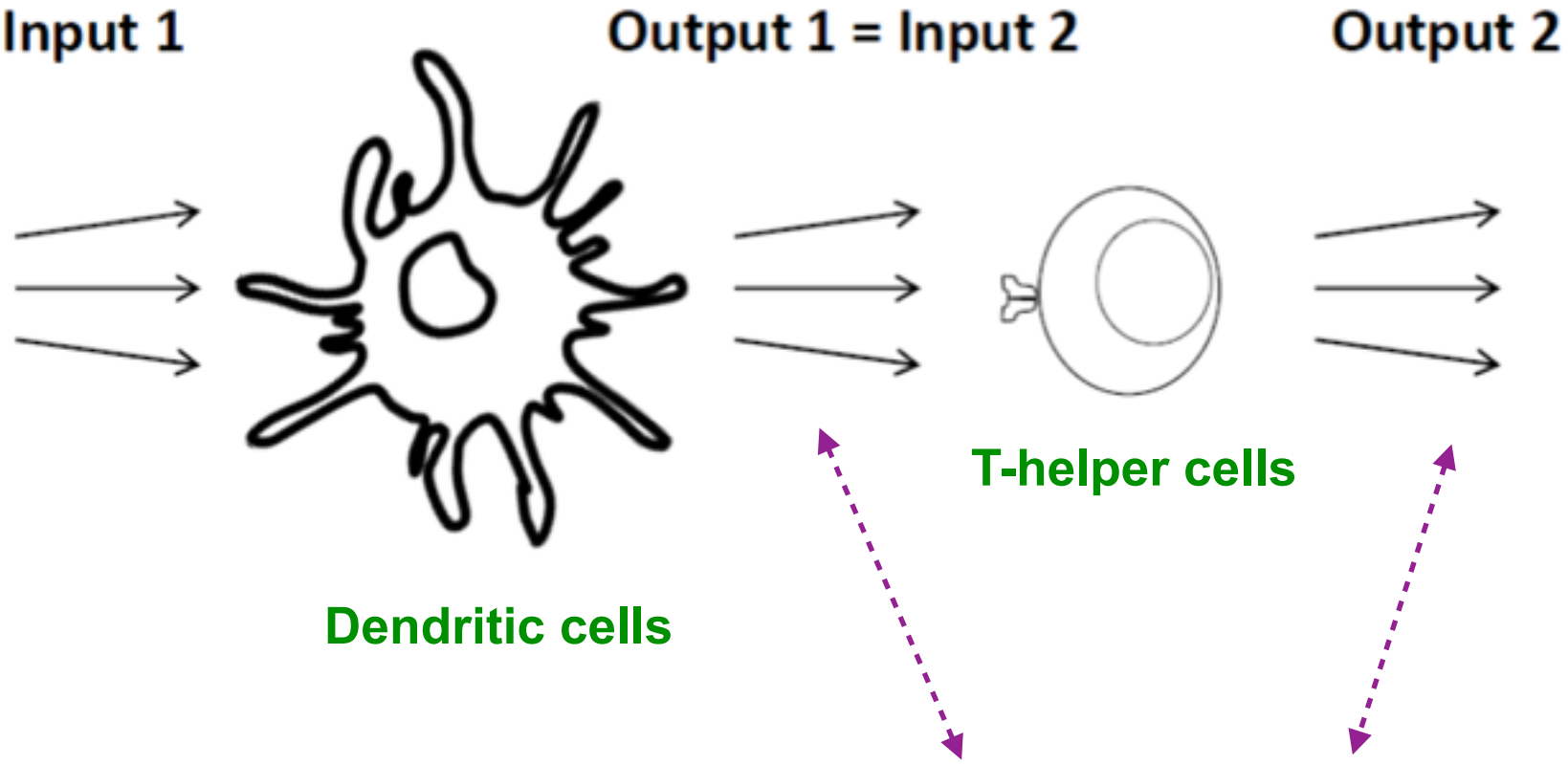
John J. O'Shea* and William E. Paul

CD4$^+$ T cells are critical for host defense but are also major drivers of immune-mediated disease. These T cells specialize to become distinct subsets and produce restricted patterns of cytokines, which are tailored to combat various microbial pathogens. Although classically viewed as distinct lineages, recent work calls into question whether helper CD4$^+$ T cell subsets are more appropriately viewed as terminally differentiated cells or works in progress. Herein, we review recent advances that pertain to this topic and the mechanisms that contribute to helper CD4$^+$ T cell commitment and plasticity. The therapeutic implications of these new findings are also considered.

Ongoing work

- Simulations of **mutants** and other perturbations (e.g. different timing for combinations of external signals)
- **Extension** of cellular model (additional pathways, transcription factors, interactions)
- Incorporation of **high-throughput datasets** (transcriptomics, proteomics) in collaboration with **Vassili Soumelis**, Institut Curie
- Towards a **multi-cellular model** - starting with DC - Th interactions

Experimental setup



Measurements (D0 ... D5)

- Surface molecules (FACS)
- Gene expression (Affy chips, RT-PCR)
- Cytokines (ELISA, Luminex, CBA)

Experimental setup

Measure of cytokine levels:

IFN-g, TNF-a, GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL21, IL-22, granzyme A , granzyme B, IFN-alpha, IFN-beta, IL-17F, IL-31, IL-33, sCD40L (22 cytokines)

Measure of surface molecules:

Co-stimulatory molecules : CD86 / CD83 / CD80 / CD40 / ICOSL / OX40L

Co-inhibitory molecules: PDL1 / PDL2

Integrins : ICAM-1 / ICAM-2 / ICAM-3 / LFA1 / VLA-4

Chemokine receptors: CCR4, CXCR3, CCR6, CCR5, CCR7

Measure of RNA expression:

Tbet, GATA-3, RORgt, FOXP3

Extension of the model (in progress)

Incorporation of novel cytokines and intracellular components

- Secreted cytokines:
  IL-5 and IL-13 (Th2 signature), IL-22 (Th22 signature), IL-6, IL-9 (Th9 signature), IL-35 (Treg signature), IL-25, IL-31 and IL-24
- Transcriptional factors:
  Bcl6 (Tfh master regulator), c-Maf (expressed in Th2 and Th17), PU.1 (Th9 signature)
- Input cytokines:
  IL-1β (Th17 polarisation in humans), IL-33, IL-25, IL-18, IL-29 and IL-36

Wiring of the novel components based on literature mining

Preliminary dynamical analysis

	RORγt	TBET	GATA3	FOXP3	IL17	IL6	IL22	IFNγ	IL4	IL5	IL13	IL9	TGFβ	IL35
Th1		■					■	■						
Th2			■						■	■	■	■		
Th17	■				■	■					■	■		
Treg				■							■		■	■
Treg Tbet+		■		■							■		■	■

Context-dependent stable states. Grey cells denote the activation of components (columns) for the corresponding stable states (rows). Simulations reproduce the canonical cell subsets Th1, Th2, Th17 and Treg (characterised by their master regulator Tbet, Gata3, RORγt and Foxp3 respectively) in response to specific cytokine environments, as well as experimentally observed hybrid subsets (Treg Tbet+) expressing several master regulators.

Outlook

- From the understanding of immune cell plasticity to **cell reprogramming**
- Ongoing project on **haematopoietic cell specification and reprogramming** (collaboration with T Graf, CRG, Barcelona)
- Collaborative project on the characterisation and modelling of **mast cell signalosome** (with B Malissen, M Daeron, J Garin, D Marguet, France)
- **Challenge**: incorporation of relevant **information from high-throughput experiments** (transcriptomics, epigenomics, proteomics, etc.) in **comprehensive and predictive dynamical models**

Selected references

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- Naldi A, Carneiro J, Chaouiya C, Thieffry D (2010). Diversity and plasticity of Th cell types predicted from regulatory network modelling. *PLoS Computational Biology* **6**: e1000912.

Collaborations & supports

- ★ **ENS (Paris)**
  - **Wassim Abou-Jaoudé**
  - Samuel Collombet
  - Jérome Feret
  - Anna Niarakis
  - Morgane Thomas-Chollier
- ★ **Institut Curie (Paris)**
  - Emmanuel Barillot
  - Isabelle Bernard-Pierrot
  - Eric Bonnet
  - Laurence Calzone
  - Philippe Hupé
  - Francois Radvanyi
  - **Vassili Soumelis**
  - **Maxime Touzot**
  - Elisabetta Volpe
  - Andrei Zinovyev
- ★ **TAGC (Marseille)**
  - Luca Grieco (=> Institut Curie)
  - Brigitte Kahn-Perlès
  - **Aurélien Naldi** (=> UNIL)
  - Jacques van Helden
- ★ **IML (Marseille)**
  - **Duncan Berenguier**
  - **Elisabeth Rémy**
- ★ **IGC (Lisboa)**
  - **Claudine Chaouiya**
  - **Jorge Carneiro**
  - **Pedro Monteiro**

Belgian Inter-university Attraction Pole

Bioinformatics and Modelling : from Genomes to Networks