Logical Modeling Peripheral T Cell Differentiation

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Acknowledgements

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  – Michael Turner
  – Lawrence Kane
  – Penelope Morel

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  – NSF (Expeditions in Computing)
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Peripheral T cell differentiation

- T cell subpopulation ratios are critical for numerous immune and auto-immune pathologies

Peripheral T cell differentiation

• T cell subpopulation ratios are critical for numerous immune and auto-immune pathologies
• Key target for immunomodulation therapy in cancer*

Dominant Role of Antigen Dose in CD4<sup>+</sup>Foxp3<sup>+</sup> Regulatory T Cell Induction and Expansion<sup>1</sup>

Michael S. Turner, Lawrence P. Kane, and Penelope A. Morel<sup>2</sup>

Naïve T cells stimulated with low Ag doses produce a high percentage of regulatory cells, which falls off as dose is increased.
Dominant Role of Antigen Dose in CD4\(^+\)Foxp3\(^+\) Regulatory T Cell Induction and Expansion\(^1\)

Michael S. Turner, Lawrence P. Kane, and Penelope A. Morel\(^2\)

Inverse correlation between Foxp3\(^+\) Treg expansion and TCR signaling via Akt/mTOR/pS6.
Key Findings

• Treg induction is determined by Ag dose
• Mechanism is T cell intrinsic
  – Observed with both iDC and mDC
  – Observed with plate-bound anti-CD3/CD28
• Inverse correlation between mTOR activation at 18h and Foxp3+ Treg at 7 days
• No exogenous TGF-β
Modeling Goals

- Determine whether known mechanisms are *sufficient* to explain experimental observations.
- Suggest *additional experiments* to identify missing mechanisms and clarifying areas of *uncertainty*.
- Identify other *early markers* of the response.
- Incorporate signals through other receptors ➔ *predictive model*. 
Rule-Based Modeling of Signal Transduction

Wiring diagram

Object-oriented model of protein

Gene names: PLCG1, PLC1
Uniprot accession number: P19174
Molecule type definition: PLCG1 (SH2_N, SH2_C, Y771∼u∼p, Y775∼u∼p, Y783∼u∼p)

Domain structure:

In the map of molecular interactions, PLCγ1 is represented with the following graph:

Phospholipase Cγ1 is an enzyme essential for T cell activation (127). It cleaves phosphatidylinositol 4,5-bisphosphate, generating the second messengers diacyl glycerol (DAG) and inositol 1,4,5-trisphosphate (IP$_3$) (128). IP$_3$ binds to receptors on the endoplasmic reticulum, leading to release of Ca$^{2+}$ (129). Itk phosphorylates PLCγ1 on Y783, which is important for activation (51,130,131). PLCγ1 binds to phosphorylated LAT (111). The

Hu, Chylek, and Hlavacek, in preparation.
Rule-Based Modeling of Signal Transduction

Hu, Chylek, and Hlavacek, in preparation.
Rule-Based Modeling of Signal Transduction

Wiring diagram

Issues
- Models are very time-consuming to construct.
- Limited knowledge about wiring.
- Lack of high-resolution data.
- Lack of measured parameters.

Hu, Chylek, and Hlavacek, in preparation.
Rule-Based Modeling of Signal Transduction

Issues
• Models are very time-consuming to construct.
• Limited knowledge about wiring.
• Lack of high-resolution data.
• Lack of measured parameters.

We did not “stand and fight” this time.

Wisdom or cowardice?

Hu, Chylek, and Hlavacek, in preparation.
A Simpler Approach
Boolean Networks

• The **state of an element** in the signaling network can be described by a **Boolean variable**, expressing that it is:
  – Active or present (on or ‘1’)
  – Inactive or absent (off or ‘0’)

• **Boolean functions**:  
  – Represent interactions between elements  
  – The state of an element is calculated from states of other elements

• The resulting network is a **Boolean network**
• Long history of applications to biology.
Logical Modeling Approach

• Generalization of Boolean – variables may have more than 2 values.
• Systematic study of the **dynamics** of large systems:
  – Depends largely on the interconnection structure
• *Does not require numerical parameters.*
• Discrete networks provide information about:
  – Multi-stationarity
  – Stability
  – Oscillatory behavior
• Highly relevant for obtaining **qualitative** measures
  – Perturbations
  – Environment
  – Alternative wiring of the network
Boolean Network Modeling Example

Biological network

Proteins: $p_1$, $p_2$, $p_3$
Boolean Network Modeling Example

Biological network

Proteins: $p_1$, $p_2$, $p_3$

Boolean network

$p_1^* = p_2 \text{ OR } p_3$
$p_2^* = \text{NOT } p_1 \text{ AND } p_3$
$p_3^* = p_1 \text{ AND NOT } p_3$
Biochemical Examples

Akt’ = PDK1 AND mTORC2

PIP3’ = PI3K AND NOT PTEN

Note that PTEN overrides PI3K here.
Boolean Models Are Logic Circuits

Boolean network

Logic circuit network

State transition diagram

\[
x_1(t+1) = x_2(t) \text{ or } x_3(t)
\]
\[
x_2(t+1) = \text{not } x_1(t) \text{ and } x_3(t)
\]
\[
x_3(t+1) = x_1(t) \text{ and } \text{not } x_3(t)
\]
Dynamics of a Boolean Model

<table>
<thead>
<tr>
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<th>$x_1x_2x_3$</th>
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Point attractor

Dynamic attractor

Attractors
Different Methods for Simulating Network Dynamics

Synchronous updates

Asynchronous updates

\[
\begin{align*}
    x_1(t+1) &= x_2(t) \text{ or } x_3(t) \\
    x_2(t+1) &= \text{not } x_1(t) \text{ and } x_3(t) \\
    x_3(t+1) &= x_1(t) \text{ and } \text{not } x_3(t)
\end{align*}
\]
Different Methods for Simulating Network Dynamics

- **Deterministic**:
  - $x_1(t+1) = x_2(t)$ or $x_3(t)$
  - $x_2(t+1) = \neg x_1(t)$ and $x_3(t)$
  - $x_3(t+1) = x_1(t)$ and $\neg x_3(t)$

- **Non-Deterministic**

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- **Synchronous updates**
- **Asynchronous updates**
Model Construction Process

Experiments

Reading and Discussion

Pathway Model Formulation and Annotation

Model Validation

Logical Model Derivation

Logical Model Simulation

Predictions

Outside experts
Model Construction Process

- Experiments
- Reading and Discussion
- Pathway Model Formulation and Annotation
- Logical Model Derivation
- Logical Model Simulation
- Predictions

Almost 1 year!
The Model

Experiments
- Reading and Discussion
- Pathway Model Formulation and Annotation
- Model Validation
  - Logical Model Derivation
  - Logical Model Simulation
- Predictions

Outside experts

~25 variables / 50 edges
The Model

~25 variables / 50 edges
Receptors:
T cell receptor (TCR)
Co-stimulation through CD28
IL-2 receptor (IL-2R)
TGFβ receptor (TGFβR)

Transcription factors:
AP-1, NFAT, NFκB, SMAD3, STAT5

Genes:
IL-2, CD25, Foxp3

Other important elements:
PTEN, PI3K, PIP3, PDK1, Akt, mTORC1, mTORC2, TSC1-TSC2, Rheb, S6K1, pS6
## Influence sets

<table>
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Logical modeling approach

Akt' = PDK1 and mTORC2
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Logical modeling approach

$\text{PIP3}' = \text{PI3K and not PTEN}$
Logical modeling decisions

• **Number of levels for element values**
  
  – TCR variable represents level of antigen stim.
    
    • No antigen (TCR_LOW = 0, TCR_HIGH = 0)
    • Low antigen dose (TCR_LOW = 1, TCR_HIGH = 0)
    • High antigen dose (TCR_LOW = 0, TCR_HIGH = 1)
TCR_LOW vs. TCR_HIGH

TCR_LOW not strong enough to overcome inhibition by PTEN.
Logical modeling decisions

- Choice between OR and AND:
  - Example:
    \[ \text{mTORC1}' = \text{Rheb} \text{ and (or?) PKC-\theta} \]
Logical modeling decisions

- Choice between AND and OR:

<table>
<thead>
<tr>
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Logical modeling decisions

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\[ mTORC1' = \text{Rheb and PKC-θ} \]

‘and’ rule means both are necessary for activation
Logical modeling decisions

- Choice between AND and OR:

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\[ \text{mTORC1}' = \text{Rheb} \]
Logical modeling decisions

- Choice between AND and OR:

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mTORC1’ = Rheb or PKC-θ

‘or’ rule means either one is sufficient for activation
Simulation setup

- **Simulation:**
  - For given initial conditions, computes system trajectory
  - Usually 20-40 steps to reach steady state

- **Scenarios (initial conditions and rules)**
  - Simulated 300 times
  - Results show the percentage of being equal ‘1’ across all runs
Model Validation

• Three main scenarios:
  1. High vs. Low antigen dose
  2. High antigen dose, then removed
  3. High antigen dose, then Akt or mTOR inhibitors added

*Results are still preliminary.*
Antigen Dose Dependence

Experimental data

Logical model results

Source: Turner et al., The Journal of Immunology, 2009, 183, 4895-4903.
Antigen Dose Dependence

Experimental data

Logical model results

Source: Turner et al., The Journal of Immunology, 2009, 183, 4895-4903.
Foxp3 vs. pS6

High Antigen Dose

Low Antigen Dose

Experiment

Model
Antigen Removal

Experimental data

Remove TCR after 18 hrs
Antigen Removal

Experimental data

Logical model results

Remove TCR after 18 hrs

with TCR signaling: 0.6%
no TCR signaling: 13.6%

% Th vs % Treg for different times:
TIME3, TIME4, TIME5, TIME6, TIME7, TIME8, TIME9, TIME10, no removal
Akt and mTOR inhibitors

Experimental data
Akt and mTOR inhibitors

Experimental data
*Source: Sauer et al., PNAS 105:7797, 2008.*
Low Antigen Trajectory
Low Antigen Trajectory
Low Antigen Trajectory
Low Antigen Trajectory

Low dose steady state
High Antigen Trajectory
High Antigen Trajectory

Suppression of PTEN allows signal to reach Akt/mTOR axis.

Could PIP3 level be a good early predictor of cell fate?
Notice that mTORC1 is activated at same time as STAT5.

If STAT5 activation happens first, Foxp3 expression can happen transiently before mTOR suppression occurs.
STAT5 vs. mTOR

Network Diagram

Circuit Diagram
STAT5 vs. mTOR

Network Diagram

Circuit Diagram

Intermediate events may be very fast.
Test effect of varying the "buffer" length.
STAT5 vs. mTOR

Circuit Diagram

Longer buffer means STAT5 wins race less often.
Role of CD25->STAT5->Foxp3

- This pathway drives *transient* Foxp3 expression at high Ag dose and *sustained* expression at low dose (in the model).
- Experiments suggest that both CD25 expression and pSTAT5 remain low in Foxp3- cells.
Role of CD25->STAT5->Foxp3

• This pathway drives *transient* Foxp3 expression at high Ag dose and *sustained* expression at low dose (in the model).
• Experiments suggest that both CD25 expression and pSTAT5 remain low in Foxp3- cells.
• Implies weak TCR stimulation may not be enough to drive CD25. *Could Foxp3 be driving CD25 instead?*
PTEN regulation

- PTEN blocks mTOR activation at low dose resulting in 100% Treg – not observed.
- Kinetics of PTEN / PIP3 could be very informative.
- Interplay with kinetics of CD25 / Foxp3 expression.
- PI3K activity increased by IL2 signaling and may partially overcome PTEN block.
Complex Interaction between mTORC1 and mTORC2

- mTORC2 activation still unclear:
  - Possible activation by PI3K or PIP3
  - Negative feedback from mTORC1 through S6K1
- Oscillations for high antigen dose
Complex Interaction between mTORC1 and mTORC2

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Complex Interaction between mTORC1 and mTORC2

- mTORC2 activation still unclear:
  - Possible activation by PI3K or PIP3
  - Negative feedback from mTORC1 through S6K1
- Oscillations for high antigen dose
- Solved by using three levels for PI3K.
mTOR role in Foxp3 expression

• Links between mTORC1 and mTORC2 and the Foxp3 expression are not well understood
  – Early mTORC1 signaling helps increase Foxp3 expression (through chromatin remodeling)
  – Prolonged mTORC1 signaling inhibits Foxp3
  – mTORC2 activation takes longer than mTORC1 activation
  – pS6 as a readout of mTORC1 activity decreases after 18 hours
  – Both mTORC1 and mTORC2 are necessary for Foxp3 inhibition
mTOR role in Foxp3 expression

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• Further Experiments: correlation between levels of mTORC1 and mTORC2 and the level of Foxp3 expression
Conclusions

• Logical modeling approach allows collaborative model development.
• Preliminary model reproduces dependence of outcome on antigen dose and duration.
• Model focuses attention on several key elements
  – Relative kinetics of CD25 / Foxp3 expression
  – Role of differential PTEN regulation
  – Possible role of Smad3
  – Negative feedback between mTORC1 and mTORC2
  – mTORC1/2 regulation of Foxp3
Future modeling steps

• Experimenting with three instead of two levels
  – Increase in number of variables is not significant in terms of simulation runtime
• Modeling of population of cells
• Exploration of the system’s sensitivity