From the two-dimensional T_{h1} and T_{h2} phenotypes to high-dimensional models for gene regulation

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Abstract

The T_{h1}/T_{h2} paradigm has been used for decades to characterize phenotypically different immune responses. Recent discoveries, e.g. T_{h17} cells are adding more dimensions to the helper T cell framework, and the T_{h1}/T_{h2} paradigm is currently being extended to include these new phenotypes. Previous mathematical models cannot easily be extended to accommodate these new phenotypes, and therefore these discoveries call for a new type of models. We devised a new model of helper T cell differentiation that describes expression of, and interactions between, the master regulators determining the phenotypic polarization of helper T cells. The model is able to describe any number of master regulators and is grounded on transcription factors binding promoter sites and binding each other. The model allows for stable switches between several different phenotypes. Furthermore, the model accounts for the kinetics of FoxP3 and GATA3 mRNA expression measured after stimulating naive helper (CD4^{+}CD45RA^{+}) T cells under various circumstances. Due to its n-dimensional character, this model may easily be applied to other developmental processes that involve master regulators.

Introduction

Helper T cells are CD4^{+} T lymphocytes that have an important role in determining the nature of the adaptive immune response. Since the 1980s, helper T cells have been classified in two major types, namely the T_{h1} or T_{h2} phenotype (1). T_{h1} cells promote cellular immunity, which is associated with anti-viral responses and tumour surveillance, whereas T_{h2} cells promote humoral responses to extracellular parasites and are involved in allergies. At a molecular level, the differentiation of a naive helper T cell towards either the T_{h1} or T_{h2} phenotype is associated with the expression of phenotype-specific transcription factors Tbet (2) and GATA3 (3), respectively. These two mutually exclusive transcription factors are ‘both necessary and sufficient’ for inducing a particular cellular phenotype and have therefore given the name ‘master regulators’ (4) to underscore their importance in cell differentiation.

The two-dimensional T_{h1}/T_{h2} paradigm has been a very successful foundation of immunology for the past 20 years. However, the recent discovery of T_{h17} cells confirmed earlier evidence that helper T cells may adopt phenotypes other than T_{h1} and T_{h2} (5). T_{h17} cells are characterized by the production of IL-17 that drives rapid neutrophil recruitment in response to bacterial and fungal infections (6, 7) and is thought to play a role in various autoimmune disorders (8).

In mice, naive helper T cell differentiation towards the T_{h17} phenotype is induced by a combination of the cytokines IL-6 and transforming growth factor (TGF) β (9), mediated by master regulators RORγt (10) and RORα (11). The induction of this phenotype in humans is different, but the resulting T cell phenotype appears to be similar (12, 13). Interestingly, TGFβ stimulation alone induces expression of another master regulator, FoxP3, which prompts the cell to adopt a regulatory (Treg) phenotype (14–16). Therefore, the T_{h17} phenotype does not only provide convincing evidence for the existence of more than two helper T phenotypes but also links the fairly separate fields of T_{h1}/T_{h2} and Treg immunology to each other. As a result, the T_{h1}/T_{h2} paradigm is currently being extended into a framework that includes at least T_{h1}, T_{h2}, T_{h17} and Treg cells (17), which functionally correspond to promoting the cellular, humoral, rapid anti-bacterial/anti-fungal and anti-inflammatory response, respectively.

To obtain more insight into helper T cell differentiation at a molecular level, mathematical models have been employed to describe the expression of the master regulators that control the different phenotypes (18–21). These models have so far only described the T_{h1} and T_{h2} phenotypes and do not accommodate newly identified lineages.
easily. We aim to extend these studies by formulating a more general framework for describing master regulators. Using the molecular biology of transcription factors, involving promoter binding and complex formation, we develop a mathematical model that intends to describe the cardinal intracellular dynamics of any number of master regulators, so that novel regulators can be incorporated as they are discovered. Furthermore, we show that the model is in agreement with previously observed master regulator kinetics in human naive CD4+CD45RA+ T cells stimulated under T2r2- and Treg-inducing conditions (22).

Methods

Mathematical model

Master regulators are initially up-regulated by external activators (E), inducing the binding of transcription factors mediating cytokine stimulation to the promoter (P₀ → P₁). Additionally, master regulator protein dimers (D) bind to the promoter (P₀ → P₂), boosting the transcription of its own mRNA (R), which in turn is translated into master regulator monomers (M). Protein monomers may be sequestered into complexes with other master regulators (N), thereby preventing their dimerization into homodimers. In principle, sequestered master regulator monomers can dissociate from the complex, but if the dissociation constant is small compared with the degradation constant of the complex, this hardly affects the results (data not shown). Master regulators in the complex are therefore taken to be directly degraded without the possibility of dissociation. This system (depicted schematically in Fig. 1) may be translated into a set of simple ordinary differential equations (Equations 1–5) using mass action kinetics:

\[
\frac{dR}{dt} = c(P_D + P_E) - d_r R \tag{1}
\]

\[
\frac{dM}{dt} = lR - d_p M - 2k_1 M^2 + 2k_{-1} D - bMN \tag{2}
\]

\[
\frac{dD}{dt} = k_1 M^2 - k_{-1} D - d_p D \tag{3}
\]

\[
\frac{dP_D}{dt} = k_2(1 - P_D - P_E)D - k_{-2} P_D \tag{4}
\]

\[
\frac{dP_E}{dt} = k_3(1 - P_D - P_E)E - k_{-3} P_E \tag{5}
\]

where c and l are transcription and translation rates, d_r and d_p are mRNA and protein degradation rates, k₁, k₂ and k₃ are association rates, k₋₁, k₋₂ and k₋₃ are dissociation rates and b is the rate of complex formation between master regulator protein monomers.

Using quasi-steady-state assumptions and the ‘conservation equation’ \( P_D + P_D + P_E = 1 \) (that bounds the promoter ‘activity’ between zero and one), these five equations can be simplified to only one equation for the expression of the master regulator. Assuming that the binding of dimers and external activators to the promoter is a fast process (i.e. \( \frac{dP_D}{dt} = \frac{dP_E}{dt} = 0 \)), we obtain a multiple substrate Michaelis–Menten term as a function for the relative activity of the promoter in terms of master regulator dimers D and external activators E:

\[
P = P_D + P_E = \frac{\frac{k_2}{k_{-2}}D + \frac{k_3}{k_{-3}}E}{1 + \frac{k_2}{k_{-2}}D + \frac{k_3}{k_{-3}}E} \tag{6}
\]

Proceeding with this expression and making quasi-steady-state assumptions for mRNA and dimers (i.e. \( \frac{dM}{dt} = \frac{dD}{dt} = 0 \)), substitution into Equation (2) yields a single differential equation describing the expression level of the master regulator.

\[
\frac{dP}{dt} = \rho \left( \frac{\alpha M^2 + \varepsilon}{1 + \alpha M^2 + \varepsilon} \right) - M(1 + \mu M + \beta N) \tag{7}
\]

with five parameters describing ‘gene production’ \( \rho = \frac{cl}{d_p d} \), ‘homodimerization’ \( \mu = \frac{k_2}{k_{-2}} \), ‘promoter affinity’ \( \alpha = \frac{k_3}{k_{-3}} \), ‘external activation’ \( \varepsilon = \frac{k_2}{k_{-2}}E \) and ‘inhibition’ \( \beta = \frac{b}{d_p} \), respectively (Box 1).

Data and fitting

The data used to fit the model were obtained from Mantel et al. (22) (Figs 4 and 5). Values were extracted using Plot Digitizer, a tool to quantify information from graphical plots. The obtained values were normalized to the maximum expression of FoxP3 and GATA3, respectively, and were fit manually using GRIND, the GReat INtegrator of Differential equations. All data sets were fit simultaneously, i.e. only the
external activation parameters $e_1$ and $e_2$ were allowed to be different between data sets, while all other parameters were kept constant. The gene production parameter $p$ was estimated from the data by observing that the average expression of FoxP3 and GATA3 increased roughly 4-fold between 0 and 96 h. Initial values were set to measurement values at $t = 0$.

Results

Developing a new model

A naive helper T cell is activated when its TCRs recognize antigen mounted on the MHC of a professional antigen-presenting cell. In response to particular co-stimulation and the local cytokine environment, the cell ultimately adopts one of several distinct helper T phenotypes (7, 24). In order to adopt the Th2 phenotype, a helper T cell needs to express high levels of the master regulator GATA3. GATA3 gene is up-regulated by transcription factors induced by TCR signaling and co-stimulation, such as nuclear factor xB (NFkB) and nuclear factor of activated T cells (NFAT) (25, 26). GATA3 is also up-regulated by specific cytokine signals, via the IL-4 receptor and Stat6, which is part of the Jak-Stat signalling pathway (27). Finally, GATA3 dimerizes and promotes its own transcription in a Stat6-independent manner (28). Likewise, the Th1 phenotype requires high expression of Tbet, which is self-activating via the extracellular cytokine route, and is also up-regulated by NFkB and NFAT. Its specific inducing cytokine is IL-12, which signals via Stat1 and Stat4 (3, 29, 30).

Consistent with the opposite phenotypes they induce, GATA3 and Tbet are typically not stably expressed in the same helper T cell. Three mechanisms have been described to accomplish mutual exclusion. Firstly, both GATA3 and Tbet repress oppositely polarizing cytokines, effectively blocking the inducing signals of the opposite phenotype (31). Secondly, up-regulation of either gene leads to epigenetic modification of the other locus, resulting in decreased transcription (31). Lastly, the TEC family kinase inducible T cell kinase (ITK) mediates the binding of GATA3 and Tbet in the nucleus forming a complex (32, 33). The GATA3–Tbet complex prevents both GATA3 and Tbet from binding to their promoter and thereby interferes with the self-activation mechanism of these master regulators. Since the last mentioned is a clear intracellular mechanistic interaction, this binding mechanism will be used in our model to describe the intracellular crosstalk between master regulators.

Using the general properties of GATA3–Tbet regulation as a paradigm, we formulate a mathematical ‘master regulator’ model (depicted schematically in Fig. 1) including (i) up-regulation upon T cell activation and cytokine stimulation, (ii) self-activation of their own transcription (i.e. positive feedback) and (iii) inhibitory interactions with other master regulators. Using quasi-steady-state assumptions, mathematical formulation of these mechanisms (see Methods) yields the following model:

$$\frac{dM}{dt} = \rho \left( \frac{M^2 + e}{1 + M^2 + e} \right) - M(1 + \mu M + \beta N)$$

(7)

where $M$ is the scaled concentration of the master regulator in the cell. A single production term, $p(M^2 + e)$, and a single degradation term, $M(1 + \mu M + \beta N)$, can be identified (Fig. 2). The production term (derived in the Methods) is a sigmoid Michaelis–Menten equation. The equation has five scaled parameters, consisting of gene production $p$, homodimerization $\mu$, promoter affinity $\alpha$, external activation $e$ and inhibition $\beta$.

Although based on mechanistic interactions, this remains a caricature model that simplifies reality: many of the details concerning activation of and interactions between master regulators are absent from the model. Potentially important post-translational modifications, such as phosphorylation, are not included because this would make the model much more complex. Such a complex model would have too many unknown parameters, and hence be less realistic, and would be difficult to understand (34). This will nevertheless be a good model if it provides new insights and if it proves to be extendable with future data.

A simple master regulator

The master regulator model describes master regulator dynamics by explicitly stating the contribution of cellular processes towards a change in concentration. Disregarding the interactions with other master regulator genes, the master
regulator concentration only depends on the production and
degradation of gene products. Due to the feedback of the
master regulator on itself, the system is expected to go to
equilibrium, i.e. a master regulator concentration where
production and degradation of gene products are equal.
Plotting production and degradation as a function of the
master regulator concentration shows that there are three
concentrations where production equals degradation
(Fig. 2). These ‘steady states’ have been numbered 0, 1
and 2 in accordance with the expression level of the master
regulator. State 0 is a resting, or naive, state with low expres-
sion of the master regulator. Once the master regulator is ac-
tivated to high expression, the system approaches state 2,
state 0. Increasing external activation can therefore lead
to a shift from the naive state to the active state 2, but after
this state has been approached, decreasing external activation
will not cause the cell to go back to the naive state. By trig-
gerating the self-activation process, a transient increase in ex-
ternal activation may act as a permanent switch that changes
the master regulator expression level from low to high.
The concentrations of the master regulator in the different
steady states obviously depend on the cellular processes.
Using a conventional Taylor expansion (see Methods),
approximations of the steady states were calculated (see
legend of Fig. 2). The level of expression in the active state
2 primarily depends on the gene production rate $\rho$ and
homodimerization rate $\mu$, since state 2 is located near the
maximum of the production curve (Fig. 2).

Two master regulators: $T_h1$ and $T_h2$

Helper T phenotypes have been shown to depend crucially
on the interaction between multiple master regulators. Using
the original $T_h1/T_h2$ paradigm as an illustration, we investi-
gate a system of two master regulators. Introducing a sub-
script to denote different master regulators and their
parameters, one can formulate a two-dimensional system as

\[
M = \frac{\rho}{1 + e} - e
\]
The gene production parameter $q$ determines the maximal production rate of a master regulator in a cell and depends on the transcription and translation rates of the cellular machinery. In order to have both naive and polarized states, the production rate $q$ should be sufficiently high (Fig. 3a) and is taken to be the same for both genes within the cell.

Phase plane analysis of Equations (8) and (9) (with in total eight parameters) shows that this system has nine different steady states, of which four are stable (Fig. 4a and b). The stable points represent the possible differentiation states that a helper T cell can attain. When Tbet and GATA3 are both lowly expressed, the T cell is naive (state 00) and resting. When either Tbet or GATA3 is highly expressed, the T cell adopts a $T_{in1}$ (state 20) or a $T_{in2}$ phenotype (state 02), respectively. When both Tbet and GATA3 are highly expressed (state 22), the T cell is active but unpolarized. Due to the mutual inhibition between Tbet and GATA3, this high co-expression normally does not occur in helper T cells, and we have searched for parameter values where the stable high co-expression state (22) is absent from the model. Bifurcation analysis of the inhibition parameter $b$ shows that one can indeed choose an inhibition rate $b$ such that state 22 is non-stable (Fig. 4a and c) or absent (Fig. 4a and d), leaving the naive, $T_{in1}$ and $T_{in2}$ phenotypes as the three stable states of the model.

Helper T cell expression of Tbet and GATA3 is induced by external signals, such as T cell activation and cytokine signalling, thereby skewing the cell towards a certain

![Fig. 4. Bifurcation analysis of the inhibition parameter $b$ in a $T_{in1}/T_{in2}$ (two-dimensional) system. The analysis shows a (pitchfork) bifurcation destabilizing the high co-expression state (22). Three phase planes show the nullclines for the indicated parameter values. The states of the system are indicated by state numbers, and stable and non-stable states are indicated by heavy and light lines (bifurcation diagrams) or by solid and open squares (phase plane diagrams). This is a symmetric system with parameter values: $q = 5$, $\mu_1 = \mu_2 = \alpha_1 = \alpha_2 = 1$, $e_1 = e_2 = 0.01$. We display phase plane diagrams for (b) $b = 0.5$, (c) $b = 1$ or (d) $b = 1.5$.](image-url)
phenotype. This is modelled by parameters $e_1$ and $e_2$ for Th1 and Th2, respectively. For sufficient and unequal external activation signals, the T cell adopts the phenotype that is most strongly activated by the external signal. When the cell is externally stimulated by a Th1 signal, the naive state disappears (Fig. 5b). As a consequence, the former location of the naive state falls within the basin of attraction of the Th1 (20) state (Fig. 5e). The cell is therefore driven towards the Th1 phenotype in response to a Th1 signal. Analogously, a Th2 signal drives a cell towards the Th2 phenotype. In response to increasing Th1 stimulation, the basin of attraction of the Th2 state shrinks and ultimately the Th2 state disappears (Fig. 5c and f). If the cell is in one of the states that is eliminated, i.e. state 00 or 02, it will go to the only stable state left which is state 20 (Fig. 5f). Effectively, helper T cell skewing blocks the cell from adopting the opposite phenotype by temporarily eliminating the other stable states from the system for the duration of the external stimulus.

An n-dimensional model

The extended framework for helper T cell differentiation currently comprises many putative master regulators that control different T helper phenotypes. When regulation of other master regulators, such as ROR$\gamma$ and FoxP3, is compared with that of Tbet and GATA3, many parallels emerge. All four are activated by NFAT and different components of the Jak-Stat pathway: ROR$\gamma$ is up-regulated by Stat3 (35) and FoxP3 by Stat3 and Stat5 (36, 37). Despite the partial overlap in specific inducing cytokine and Jak-Stat signals, the expression of all four master regulators remains mutually exclusive with respect to each other. Intracellular self-activation has not been demonstrated for ROR$\gamma$ and FoxP3, but both reinforce their own expression at least via the extracellular cytokine route (7, 38).

To accommodate these master regulators, the two-dimensional master regulator model can be expressed as an n-dimensional framework. The maximum number of steady states depends on the total number of master regulators (i.e. $n$) in the model: three steady states per master regulator (resting, saddle and active) allows for a maximum of $3^n$ possible steady states, of which $2^n$ states are stable. All genes are transcribed and translated by the same molecular machinery; hence, all genes are assigned the same productivity $q$, making the total number of parameters in such a model is $1 + 2^n + n^2$.

To model each of these master regulators, they are each denoted by $M_i$ for $i = 1$ to $n$:

$$\frac{dM_i}{dt} = \rho \left[ \frac{\alpha_i M_i^2 + e_i}{1 + \alpha_i M_i^2 + e_i} \right] - M_i \left( 1 + \sum_{j=1}^{n} B_{ij} M_j \right)$$

where $\alpha_i$ is the self-activation parameter, $e_i$ is the external activation parameter and $B_{ij}$ is a matrix of complex formation parameters. This notation conveniently integrates parameter

![Fig. 5. Phase plane analysis of the Th1/Th2 (two-dimensional) master regulator model. The upper panels (a, b and c) show nullclines and steady states, with the corresponding separatrices (panels d and e) or exemplary trajectories (panel f, grey lines) shown below. The black lines are the $\frac{dM_i}{dt}=0$ nullclines and the grey those of $M_i$ (panels a, b and c). The naming convention for states was chosen such that the four stable states (solid squares) have only even numbers (0 and 2) in their state name, whereas the five non-stable states (open squares) also contain a 1 in their state name. The vector field is indicated by arrows in each qualitatively different area. Parameter values: $\rho = 5$, $\beta = 1$, $\mu_1 = \mu_2 = \alpha_1 = \alpha_2 = 1$ and $e_2 = 0.01$. In panels a and d, we set $e_1 = 0.01$ to show all possible steady states; in panels b and e, we set $e_1 = 0.02$ (mild Th1 skewing) and $\beta = 1$ and in panels c and f, we set $e_1 = 0.1$ (strong Th1 skewing) to show the effect of asymmetric external stimulation.](image-url)
\(\mu_i\) and \(\beta_{ij}\) into a single matrix; the homodimerization parameters \(\mu_i\) are the diagonal of this matrix, and inhibition parameters \(\beta_{ij}\) form all off-diagonal elements. As yet, there is hardly any information about the exact topology of this matrix, but this is expected to change in the near future.

**Accounting for FoxP3/GATA3 data**

To illustrate the application of the model to data, we searched for parameter values that describe master regulator kinetics in human CD4+CD45RA+ T cells. These data, published by Mantel et al. (22), comprise FoxP3 and GATA3 mRNA expression levels measured in naive Th, after stimulation under either Treg-skewing conditions (plate bound aCD3-aCD28 + TGF\(\beta\), 5 ng ml\(^{-1}\)), Th2 skewing (plate bound aCD3-aCD28 + IL-4, 25 ng ml\(^{-1}\)) or Th0 (stimulation with plate bound aCD3-aCD28 only). FoxP3 and GATA3 expressions were measured at 0, 2, 6, 24 and 96 h after stimulation. For details concerning the data and the exact experimental set-up, we refer to the original publication by Mantel et al. (22).

Taking the limited number of available data points and the relatively large number of model parameter into account, we searched manually for an illustrative fit and refrained from performing a full non-linear parameter fit. To mark the transition from a resting to an active cell, we let gene production \(\rho\) increase over time (see the legend of Fig. 6), as naive T cells have been shown to slowly increase their rates of transcription and translation in response to activation (39).

Increasing the gene production parameter \(\rho\) facilitates the cell to approach the high-expression steady states (Fig. 3a). The kinetics of master regulators FoxP3 and GATA3 in this system are described reasonably well by our model (Fig. 6).

The model clearly accounts for the establishment of a dominant master regulator under certain polarizing conditions: FoxP3 for Treg conditions (Fig. 6a) and GATA3 for Th2 conditions (Fig. 6b). Furthermore, for the same parameter

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**Fig. 6.** The master regulator model agrees with experimental data of Mantel et al. (22). Th were stimulated under three different conditions: Treg (a), Th2 (b) and Th0 (c). FoxP3 (\(M_1\)) and GATA3 measurements (\(M_2\)) are depicted as squares and circles, respectively, with error bars indicated. For visualization reasons, the data are plotted slightly offset with respect to time; all data were collected at 0, 2, 6, 24 or 96 h. In (d), increasing IL-4 concentration is modelled using the parameter \(e_2\) as a bifurcation parameter, and the data are plotted similarly as in (a–c). Parameter values: \(\rho=0.3+0.01t\) (\(t\) is time (hours)), \(\mu_1=\mu_2=0.01\), \(\alpha_1=4.5\), \(\alpha_2=4.0\) and inhibition \(I=2\). In panel a, we set \(e_1=1\) and \(e_2=0.25\); in panel b, \(e_1=0.4\) and \(e_2=2\); in panel c, we set \(e_1=0.5\) and \(e_2=0.5\). In panel d, we set \(\rho\) to 1.7 (i.e. we fix time at \(t=120\)) in accordance with the experiment readout time of 5 days; \(e_1=0.6\), \(e_2=([\text{IL}-4\text{ (pg ml}^{-1}\text{)}])/2).
values, the model describes the dynamics of these master regulators stimulated only with activation signals, i.e., in a non-polarizing Th0 environment (Fig. 6c). In addition to describing the dynamics of FoxP3 and GATA3 in time course experiments, the model accounts for the response of naive T cells to conflicting differentiation signals. Naive Th were activated and stimulated with TGFβ and different concentrations of IL-4. Expressions of FoxP3 and GATA3 were quantified after 5 days of stimulation. To implement these conditions in the model, the external activator IL-4 (parameter $c_2$) was varied and the model was observed at $t = 120$, i.e., after 5 days. The model should not be expected to fit this data perfectly, because it remains a caricature model missing (for instance) regulatory elements in the signal transduction pathway that connects extracellular IL-4 concentration to GATA3 expression.

Discussion

The mathematical model presented here describes the differentiation of helper T cells by the expression of master regulators, which are transcription factors that are both necessary and sufficient for the induction of a certain cellular phenotype (4). We have shown that a bistable master regulator can be switched from a state of low to high expression by extracellular cytokine stimulation. Co-expression of master regulators can be prevented by strong mutual inhibition through complex formation. Transient helper T cell skewing prevents the cell from differentiating towards the opposite phenotype by temporarily eliminating the opposite stable state from the system. Once polarized, the cell resists phenotype switching and hence displays phenotype memory. These properties are sufficient to explain the differentiation of a T cell into several exclusive phenotypes.

Previous models have described only one (18) or two (19–21, 40) master regulators. Our model differs from these models by using a more mechanistic rather than a phenomenological description of transcription factor binding. Firstly, using dimerization of transcription factors and binding to DNA, we show that the activation term can be approximated by Michaelis–Menten kinetics describing enzyme–multiple substrate interactions. Furthermore, mutual inhibition is described mechanistically by master regulator sequestration into a nuclear complex rather than a generic inhibition term. Despite the fact that our model is more mechanistic, it is also more simple than earlier models, and yet we obtain similar results in one and two dimensions. The ability of our extended model to describe any number of master regulators allows for more complex networks of genes to be analysed and permits incorporation of newly discovered phenotypes and their master regulators.

To illustrate the model's ability to describe real data, we show that the model can account for data published by Mantel et al. (22). Experiments that would allow us to fit this model to data are rare, because studies typically perform measurements at one or two time points or provide qualitative rather than quantitative experimental readouts. To our knowledge, this is the first attempt to describe helper T cell transcription factors over time. The model that we present disregards much of the known detail about induction and propagation of master regulator genes, but the agreement between model and data suggests that we have may succeeded in capturing the essence of the regulation of these genes in this caricature model. Strictly speaking, a model is always incorrect, because it only approximates reality. However, a good model allows one to focus on the important processes that primarily determine master regulator dynamics. In other words, the model developed here is ‘no more, but no less, than a way of thinking clearly’ (34).

Further confirmation and testing of the model would need detailed data about master regulator kinetics in time. Using a data set that measures all master regulators simultaneously in response to different (and if possible, conflicting) stimuli, one could estimate master regulator-specific parameters. Although a good fit to experimental data by no means proves that a model is accurate, it is a minimal requirement for a correct model. As a test of the framework of the model, additional biochemical studies into the mechanism of inhibition between the master regulators are required. Indeed, it was shown that retrovirus-mediated up-regulation Tbet or GATA3 in IL-4 and IFNγ knockout mice is sufficient to inhibit FoxP3 (41), which is consistent with the inhibition by complex formation. Furthermore, an interaction between FoxP3 and RORγt was recently shown to be an inhibitory mechanism in Th differentiation (42).

The application of this model is not limited to T cell biology or even immunology. The generic features described in the model can be found in many other developmental systems. Indeed, the master regulator has been adopted for describing developmental processes in general (43, 44), and this mathematical model could similarly be applied to these gene regulatory networks involved in cellular differentiation.

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Abbreviation

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<tr>
<td>TGF</td>
<td>transforming growth factor</td>
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<tr>
<td>NFκB</td>
<td>nuclear factor κB</td>
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<td>NFAT</td>
<td>nuclear factor of activated T cells</td>
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