A Mathematical Model of the NFAT Genetic Circuit

We describe here in detail the theoretical underpinnings of our mathematical model, aiming to characterize the response of a generic cell to an external stimulus. The results examined in the main text of this paper led us to conclude that the failure of the NFAT genetic circuit might cause the wide variety of phenotypes observed in Down Syndrome.

We set up a system of ordinary differential equations, describing the time evolution of all the core variables constituting the Calcineurin/NFAT pathway (See text, Fig. 4a for a schematic description of the pathway). Increase or decrease over time of a specific concentration is achieved in the model by gain and loss terms, coupling the equations to each other and making it necessary to perform a numerical study.

Calcineurin Activation

The Calcineurin/NFAT pathway is activated by an increase in the concentration of intracellular calcium $[Ca^{2+}]_i$. Calcineurin (Cn) is allosterically switched from an "off" state to either a "free" or "occupied" state. The "free" state is achieved first, with a certain probability that Cn dissociates from the Ca^{2+}/Calmodulin complex and goes back to the "off" state. Activated Cn in the "free" state can swtich to the "occupied" state if it binds to the competitive inhibitor DSCR1. There is a certain probability that DSCR1 dissociates from Cn, switching it back to the "free" state. This description of Cn is summarized in the following equations:

$$\frac{d\,Cn^{free}(t)}{dt} = +K^{on}_{Ca}(t)\,Cn^{off}(t) - K^{off}_{Ca}\,Cn^{free}(t) - \frac{d\,Cn^{occ}(t)}{dt} \quad (1)$$

$$\frac{d\,Cn^{occ}(t)}{dt} = +K^{occ}_{DSCR1}\,Cn^{free}(t)\,DSCR1(t) - K^{free}_{DSCR1}\,Cn^{occ}(t) \quad (2)$$

$$\frac{d\,Cn^{off}(t)}{dt} = -\frac{d\,Cn^{free}(t)}{dt} - \frac{d\,Cn^{occ}(t)}{dt} \quad (3)$$

In equation (1) $K^{on/off}_{Ca}$ are the activation/deactivation rates for Cn. The activation rate depends on the Ca^{2+} signal, and its time dependence depends on the signal profile. The spontaneous deactivation rate is assumed to be constant. In equation (2) $K^{occ/free}_{DSCR1}$ are the constant occupation/liberation rates for Cn interacting with DSCR1. Equation (3) can be solved immediately, leading to $Cn^{off}(t) = Cn^{eq} - Cn^{free}(t) - C^{occ}(t)$, where Cn^{eq} is the total, time-independent amount of Cn.

NFAT Transport and Feedback

The previous equations describe the available amount of Cn that can dephosphorylate NFAT. When NFAT$_\wedge$ is dephosphorylated, it translocates to the nucleus and acts as a transcription factor, initiating two feed-back loops. NFAT$_\wedge$ activates transcription of itself (in the case of NFATc1 and NFATc4), which represents a positive feedback loop. It also activates transcription of the Cn inhibitor DSCR1, which represents a negative feedback loop. At the same time, DYRK1a phosphorylates

nuclear NFAT$_n$ priming it for subsequent phosphorylation by GSK-3 and nuclear export. Cytoplasmic NFAT$_c$ accumulates. Eventually, the signal from the external stimulus attenuates and the system returns to its initial steady-state, as excess NFAT$_c$ is degraded through normal protein turnover. This description of NFAT$_c$ is summarized in the following equations:

$$\frac{d\,NFAT^{in}(t)}{dt} = K^{in}_{NFAT}\,Cn^{free}(t)\,NFAT^{out}(t) - (K^{out}_{DYRK1a} + K^{out}_{Export})\,NFAT^{in}(t) \quad (4)$$

$$\frac{d\,NFAT^{out}(t)}{dt} = -\frac{d\,NFAT^{in}(t)}{dt} + K^{loop}_{NFAT}\,NFAT^{in}(t - t^{NFAT}_{lag}) - K^{deg}_{NFAT}\,[NFAT^{out}(t) - NFAT^{eq}] \quad (5)$$

$$\frac{d\,DSCR1(t)}{dt} = +K^{prod}_{DSCR1}\,NFAT^{in}(t - t^{DSCR1}_{lag}) - K^{deg}_{DSCR1}\,[DSCR1(t) - DSCR1^{eq}] \quad (6)$$

In equation (4) K^{in}_{NFAT} is the rate per unit concentration for NFAT nuclear import, while K^{out}_{DYRK1a} and K^{out}_{Export} represent the rate of NFAT nuclear export determined by the presence of DYRK1a and of independent kinases, as DYRK1a is not the exclusive nuclear NFAT priming kinase (unpublished observation). We assume that these rates are constant and that DYRK1a is not regulated by the pathway itself. Equation (5) represents the time variation of the NFAT concentration in the cytoplasm, governed by the rate K^{loop}_{NFAT} of auto-regulation and the rate K^{deg}_{NFAT} of degradation in the cytoplasm due to normal protein turnover. In the cytoplasm NFAT is initially in equilibrium at the value $NFAT^{eq}$. There is an intrinsic time lag for transcription and translation of NFAT to occur, denoted by t^{NFAT}_{lag}. Equation (6) describes the production of the Cn inhibitor DSCR1, controlled by the kinetic constants K^{prod}_{DSCR1} for production and K^{deg}_{DSCR1} for degradation due to normal protein turnover. In equilibrium, the concentration of DSCR1 is at the value $DSCR1^{eq}$. Within our model, the lag time t^{DSCR1}_{lag} for transcription/translation of DSCR1 is assumed to be substantially larger than that for the NFAT loop.

Gene Dosage

The action of DYRK1a is controlled by the constant K^{out}_{DYRK1a}, which implicitly contains the concentration of DYRK1a itself. Therefore it is always multiplied by the quantity $\delta^{dosage}_{DYRK1a} = 1$ or 1.5 which controls its gene dosage. Analogously for DSCR1, the constant K^{prod}_{DSCR1} regulating DSCR1 production, is multiplied by $\delta^{dosage}_{DSCR1} = 1$ or 1.5, thereby specifying its gene dosage. By independently changing the values of δ^{dosage}_{DYRK1a} and δ^{dosage}_{DSCR1} it is possible to simulate various degrees of partial trisomy, leading to circuit dysregulation of varying severity.

Steady state

The above equations allow the cell to be considered to be in an equilibrated steady state, able to maintain all the relevant variables constant in time. The steady state is controlled by a constant small background Ca^{2+} signal. In the unstimulated steady state, Cn is mostly inactive, but a small proportion is activated to a constant level by the background Ca^{2+} signal. NFAT is mostly in the cytoplasm, with a small amount in the nucleus due to the small activated fraction of Cn. This implies that the feed-back mechanisms present in the cell, and therefore in the model, are active at all times.

$NFAT_\Lambda$ activates itself while also allowing for basal DSCR1 production, which in turn blocks part of the active Cn from dephosphorylating NFAT. A large transient Ca^{2+} signal disturbs the steady state equilibrium and activates the genetic circuit. The circuit is dynamically active for a time that is typically much longer than that of the signal itself, but as the signal returns to its background value, all the variables slowly relax and the system returns to its equilibrium steady state.

In order to find the steady-state values of the different dynamic variables it is sufficient to set the right hand sides of equations (1-6) to zero, leading to

$$K^{on}_{Ca,0}\, Cn^{off}_0 - K^{off}_{Ca}\, Cn^{free}_0 \;=\; 0 \tag{7}$$

$$K^{occ}_{DSCR1}\, Cn^{free}_0\, DSCR1_0 - K^{free}_{DSCR1}\, Cn^{occ}_0 \;=\; 0 \tag{8}$$

$$Cn^{off}_0 + Cn^{free}_0 + C^{occ}_0 - Cn^{eq} \;=\; 0 \tag{9}$$

$$K^{in}_{NFAT}\, Cn^{free}_0\, NFAT^{out}_0 - (K^{out}_{DYRK1a} + K^{out}_{Export})\, NFAT^{in}_0 \;=\; 0 \tag{10}$$

$$K^{loop}_{NFAT}\, NFAT^{in}_0 - K^{deg}_{NFAT}\, (NFAT^{out}_0 - NFAT^{eq}) \;=\; 0 \tag{11}$$

$$K^{prod}_{DSCR1}\, NFAT^{in}_0 - K^{deg}_{DSCR1}\, (DSCR1_0 - DSCR1^{eq}) \;=\; 0 \tag{12}$$

Parameter Choice

The values of all the parameters have been fixed with the aim of illustrating the biological content of the model. In fact, most cellular quantities are expected to vary significantly, in some cases by two orders of magnitude, depending on the cell (tissue) type and on the developmental stage one wishes to examine. In other words, they are space- and time-dependent.

As organ and tissue development is often initiated from a single cell or small subpopulation of cells that may receive varying levels of a given stimulus within embryonic tissue, estimating resting concentrations of various cellular components in these initiating cells based on experimental data from a necessarily larger population of cells is likely to be misleading. Therefore, we have designed our model to encompass a range of possible starting values for each component of the system to examine whether increased dosage of DYRK1a and DSCR1 can affect the output of the NFAT genetic circuit over this range. For instance, Cn concentrations vary from about 5000 to 200000 molecules/cell in B Lymphocytes and neurons, respectively, NFATc1 concentrations vary from about 1000 to 10000 molecules/cell in active and non-active T cells and NFATc4 concentrations vary from about 200 to 1000 molecules/cell in cortical neurons. In Thymocytes DSCR1 concentrations have an upper limit of $2\mathrm{x}10^5$ molecules/cell, while in neurons the upper limit is 10^6 molecules/cell. DYRK1a concentrations are in the range of 1000-2500 molecules/cell in thymocytes and neurons, respectively. These experimental values have been determined using semi-quantitative Western blots using recombinant proteins as a standard. Model parameters have been scaled from the above values and the results presented in Fig. 4b and d and in this supplemental discussion are prototypical, in the sense that they illustrate inequivocally the extreme cases of unstable and stable responses to the Calcium stimulus.

Clearly, if increased dosage of DYRK1a and DSCR1 inhibited NFAT activity below the threshold activation value for all NFAT-regulated genes, development would not proceed past very early embry-

onic stages (as observed in Cnb1-/-, NFATc1-/-, NFATc3/c4 DKO, and NFAT c2/c3/c4 TKO mice) and trisomy 21 would be incompatible with life. While Down Syndrome comprises a constellation of developmental phenotypes of varying severity, clearly some level of NFAT activation is possible in developing trisomy 21 tissues, thus the dramatic phenotypes are likely the result of subtle variations in NFAT activity in individual cells or subpopulations of cells in the developing embryo.

Numerical Results

To illustrate the response of the circuit within our model we chose the form $K_{Ca}^{on}(t) = K_{Ca}^{on} f_{Ca}(t)$ with $f_{Ca}(t) = f_0 + (1 - f_0)[\theta(t)\theta(\bar{\tau} - t) + 0.5\theta(t - \bar{\tau}) \exp(-(t - \bar{\tau})/\tau_{Ca})]$[1] for the Ca^{2+} impulse, with $\bar{\tau} = 2$ minutes and $f_0 = 2.0 \times 10^{-2}$. The values of all model parameters are listed in the table below. As a reference we used the "Unstable" case from Fig. 4b. All other cases have been obtained by changing only the values indicated.

Quantity	Fig. 4b (Unstable)	Fig. 4d (Stable)	Setup 1	Setup 2
Cn_0^{off}	1.0×10^{-7}			$\mathbf{5.0 \times 10^{-9}}$
$NFAT_0^{out}$	1.0×10^{-9}			
$DSCR1_0$	2.0×10^{-9}			
K_{Ca}^{on}	2.0		**1.0**	
τ_{Ca}	3 hours		**1.5 hours**	
K_{Ca}^{off}	5.0			
K_{DSCR1}^{occ}	2.0×10^{12}			
K_{DSCR1}^{free}	1.0×10^{2}			
K_{NFAT}^{in}	1.0×10^{8}			
K_{DYRK1a}^{out}	2.7×10^{-2}	$\mathbf{1.0 \times 10^{-5}}$		
K_{Export}^{out}	3.0×10^{-3}	$\mathbf{3.0 \times 10^{-2}}$		
K_{NFAT}^{loop}	4.0×10^{-3}			
K_{NFAT}^{deg}	2.0×10^{-2}			
K_{DSCR1}^{prod}	2.0×10^{-1}	$\mathbf{2.0 \times 10^{-3}}$		
K_{DSCR1}^{deg}	5.0×10^{-2}			
t_{lag}^{NFAT}	1 hour			
t_{lag}^{DSCR1}	4 hours			

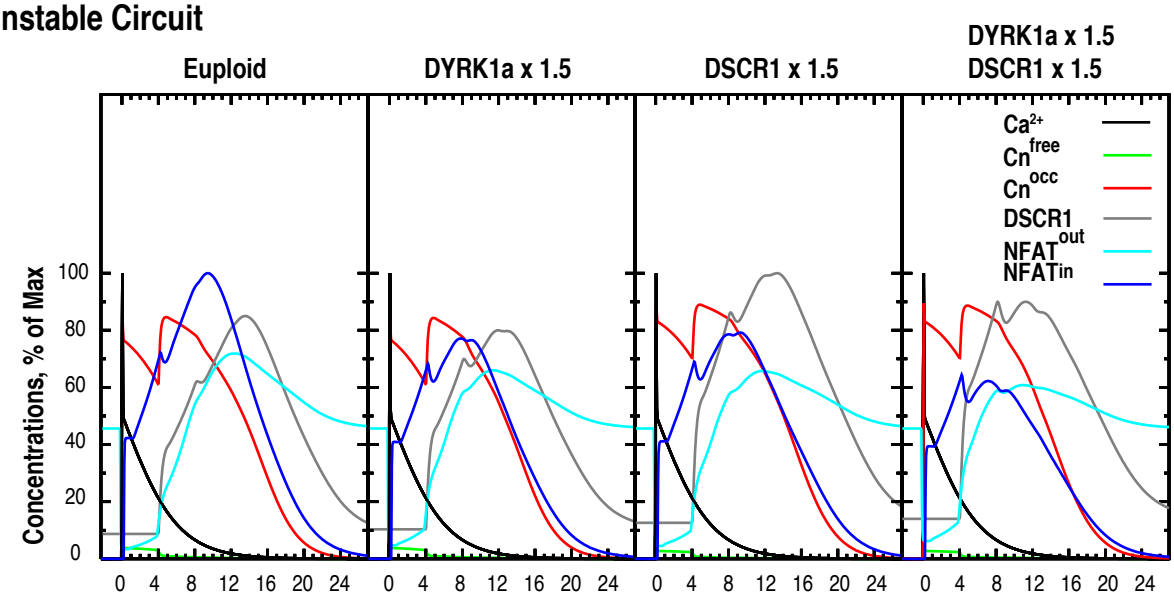
The initial values of the variables Cn_0^{free}, Cn_0^{on}, $NFAT_0^{in}$, $NFAT^{eq}$, $DSCR1_0^{eq}$, have been obtained by solving equations (7-12) at equilibrium, expressing the variables in terms of combinations of quantities from the above table.

Below we plot the results of our simulations obtained according to the values in the table. The first case labelled "Unstable" is identical to the one in Fig. 4b. For completeness we added here the curves for Cn^{free}, Cn^{occ} and DSCR1. The second case labelled "Setup 1" is obtained by simply using a weaker and shorter Ca^{2+} stimulus. Here the genetic circuit is less sensitive to DSCR1 gene

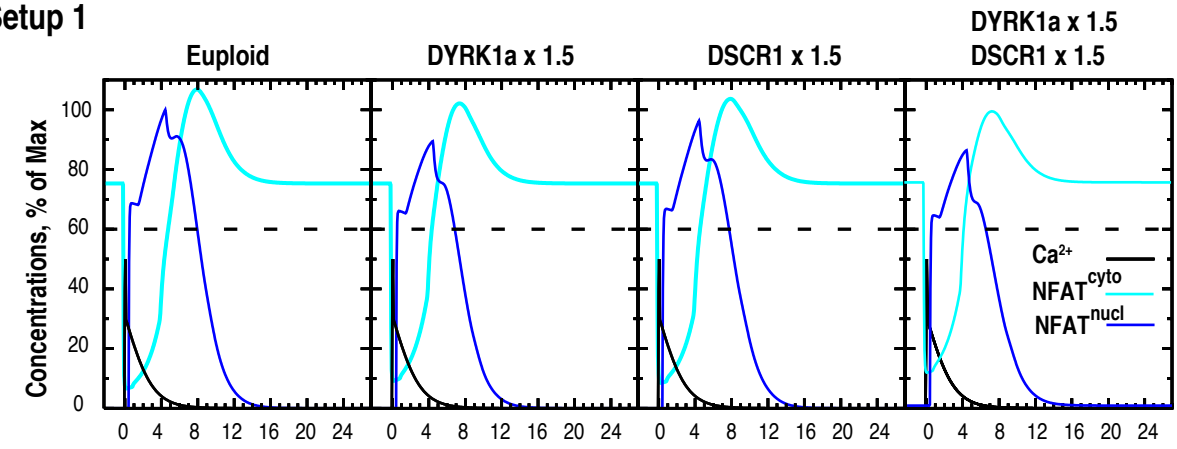
[1] $\theta(t) = 0$ for $t < 0$ while $\theta(t) = 1$ for $t \geq 0$

dosage because the stimulus attenuates before a significant amount of DSCR1 can accumulate. The third case labelled "Setup 2" is obtained by substantially reducing the initial concentration of Cn. The circuit is nevertheless impaired despite a markedly different initial state. In all simulations we indicated the presence of a sample threshold for activation of NFAT target genes, at $60\,\%$. With this we wish to indicate that disruption of the genetic circuit may be achieved only for some of the target genes, those that require a higher activation threshold. Others may still be activated, albeit to a lesser degree, and their activity may or may not give rise to any phenotypic change.

Unstable Circuit



Setup 1



Setup 2