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0.1 Mathematical Modeling

0.1.1 Utility of the model

The purpose of our model was to test our understanding of the dynamics of the gene expression response to transient IFNγ exposure. Taking as a null hypothesis the simplest model of gene expression governed by the central dogma of molecular biology, we asked when IFNγ-dependent gene expression peaked. The central dogma suggests that under these circumstances, gene expression would peak at the time the signal was abolished (in our case, 5h). This stood in contradiction to our observation of persistent mRNA transcription post IFNγ exposure. Given our experimental results, which suggested that IFNγ was captured and released by cells, we added a non-signaling, IFNγ-capture site to our model with the goal of testing whether this non-signaling capture site could recapitulate our experimental observations. Once this component was added, we used the model to constrain the values of its biochemical parameters. By testing biochemical parameter values *in silico*, we were able to generate hypotheses that were subsequently tested using targeted experiments. Finally, we used this refined model with experimentally-determined biochemical parameters, to test whether the mechanism of cytokine catch-and-release could explain our key experimental observations, namely the long-term dynamics of pSTAT1 and mRNA.

0.1.2 Summary of modeling

First, we describe the process of STAT1 phosphorylation upon exposure to IFNγ. We show that this process follows a typical Hill function dose response with Hill coefficient $= 1$ and very fast ($\sim$ seconds) dynamics. Next, we model the dynamics of IFNγ catch-and-release, and the resulting dynamics of pSTAT1. The model was designed to recapitulate our experimental setup, namely: (1) initial exposure to IFNγ, (2) three wash steps consisting of approximately 20 minutes total, and (3) long-term culture. We show that the catch-and-release mechanism must have relatively slow ($\sim$ hours) dynamics to account for our measured pSTAT1 and mRNA dynamics. This prediction is corroborated by direct measurements of PS-dependent IFNγ capture and release from the cells. Lastly, we incorporate these into a model of mRNA transcription and model the dynamics of mRNA over the period of our experiment.

0.1.3 phosphorylation of STAT1

The effect of IFNγ on mRNA transcription is taken into account through activation of the transcription factor STAT1: upon binding of IFNγ to its receptor, STAT1 is phosphorylated by Janus Kinases (JAK), dimerizes, and translocates to the nucleus. These processes are summarized by the following set of coarse-grained equations,

$$\begin{cases} \frac{d[\text{IFN}\gamma R_c]}{dt} = k_{\text{on}}^R \cdot [\text{IFN}\gamma R] \cdot [\text{IFN}\gamma] - k_{\text{off}}^R \cdot [\text{IFN}\gamma R_c], \\ \frac{d[\text{IFN}\gamma R]}{dt} = -\frac{d[\text{IFN}\gamma R_c]}{dt}, \\ \frac{d[\text{pSTAT1}]}{dt} = k_{\text{phos}} \cdot [\text{STAT1}] \cdot [\text{IFN}\gamma R_c] - k_{\text{deg}} \cdot [\text{pSTAT1}], \\ \frac{d[\text{STAT1}]}{dt} = -\frac{d[\text{pSTAT1}]}{dt}, \end{cases} \tag{1}$$

where IFNγR is the IFNγ receptor, IFNγR_c is the complex of IFNγ and its receptor, k_{on}^R and k_{off}^R are the kinetic on- and off- rates of the IFNγR_c complex formation, and k_{phos} and k_{deg} are the rates of phosphorylation of STAT1, modeled here as an interaction between STAT1 and the IFNγ-IFNγ receptor complex, and dephosphorylation and pSTAT1.

This phosphorylation occurs rapidly upon exposure of the cells to the cytokine, reaching a steady state within 5 minutes (Figure S4F). Following treatment with a JAK inhibitor, pSTAT1 levels in the system rapidly return to baseline levels (Figure S2A). Given that the time scales of the effects we observe in the system are on the order of magnitude of days, for ease of computation, the dynamics of pSTAT1 up- and down- regulation can be neglected and the system is assumed to reach steady state instantaneously.

Using simple conservation of mass:

$$\begin{aligned}[\text{IFN}\gamma R_{\text{total}}] &= [\text{IFN}\gamma R] + [\text{IFN}\gamma R_c],\\ [\text{STAT1}_{\text{total}}] &= [\text{STAT1}] + [\text{pSTAT1}],\end{aligned} \tag{2}$$

where IFNγR_{total}, and STAT1$_{\text{total}}$ are the total levels of IFNγ receptor and STAT1, respectively. From these equations, we calculate the steady-state of IFNγR_c and pSTAT1 for a given dose of IFNγ:

$$\begin{aligned}[\text{IFN}\gamma R_c] &= [\text{IFN}\gamma R_{\text{total}}]\frac{k_{\text{on}}^R\cdot[\text{IFN}\gamma]}{(k_{\text{on}}^R\cdot[\text{IFN}\gamma])+k_{\text{off}}^R} \propto \frac{1}{1+\frac{K_D^R}{[\text{IFN}\gamma]}}\\ [\text{pSTAT1}] &= \frac{[\text{IFN}\gamma R_{\text{total}}][\text{STAT1}_{\text{total}}]k_{\text{phos}}k_{\text{on}}}{[\text{IFN}\gamma R_{\text{total}}]k_{\text{on}}k_{\text{phos}}+k_{\text{deg}}k_{\text{on}}}\left(\frac{[\text{IFN}\gamma]}{[\text{IFN}\gamma]+\frac{k_{\text{deg}}k_{\text{off}}}{[\text{IFN}\gamma R_{\text{total}}]k_{\text{on}}k_{\text{phos}}+k_{\text{deg}}k_{\text{on}}}}\right) \propto \frac{1}{1+\frac{\text{EC}_{50}}{[\text{IFN}\gamma]}}.\end{aligned} \tag{3}$$

where $K_D^R = \frac{k_{\text{off}}^R}{k_{\text{on}}^R}$.

The dose dependance of pSTAT1 on IFNγ concentrations is expected to follow a Hill function with coefficient 1. Figure S4A shows that cells indeed follow such a dose response curve with $\text{EC}_{50} \approx 3pM$:

$$\text{pSTAT1}([\text{IFN}\gamma]) \propto \frac{1}{1+\frac{\text{EC}_{50}}{[\text{IFN}\gamma]}}. \tag{4}$$

0.1.4 Dynamics of IFNγ catch-and-release

Mass action kinetics were used to model binding of IFNγ to its receptor and to the cell. Initially, a constant dose of IFNγ is supplied to the system. Once cells are washed, the initial concentration of IFNγ outside the cell is zero, then release of IFNγ from the cell drives persistent signaling through the IFNγ receptor until it is consumed to a quantity below the threshold of signaling. IFNγ decays by endocytosis, molecular degradation, and dilution over proliferating cells. These processes are combined into one *removal rate* - k_{removal}. Our model can be summarized using the following system of ordinary differential equations.

$$\begin{cases}\text{pSTAT1}([\text{IFN}\gamma]) \propto \frac{1}{1+\frac{\text{EC}_{50}}{[\text{IFN}\gamma]}},\\ \frac{d[X-\gamma]}{dt} = k_{\text{catch}}^X\cdot[X]\cdot[\text{IFN}\gamma] - k_{\text{release}}^X\cdot[X-\gamma],\\ \frac{d[\text{IFN}\gamma]}{dt} = -k_{\text{catch}}^X\cdot[X]\cdot[\text{IFN}\gamma] + k_{\text{release}}^X\cdot[X-\gamma] - k_{\text{removal}}[\text{IFN}\gamma],\end{cases} \tag{5}$$

where X is the IFNγ non-signaling cell capture site, $X-\gamma$ is the complex of IFNγ and X, k_{catch}^X and k_{release}^X are the kinetic catch and release rates of the $X-\gamma$ complex formation. Our evidence suggests that IFNγ enters and exits the cell after it adheres to PS on the cell surface. We coarse-grained this interaction by modeling it as a single-step process. We use this catch-and-release model to generate the dynamics of IFNγ as a function of time, and from that calculate pSTAT1(t), the dynamics of phosphorylated STAT1 (Figure 4C).

By comparing these solutions to experimental measurements (Figure 2B) we learn that this catch-and-release mechanism must have a release rate in the range $k_{\text{release}}^X \approx 5\times10^{-6}$ - 2×10^{-5} $[s^{-1}]$ (mean interaction time ≈ 7.5 hours), orders of magnitude lower than that of the IFNγ receptor - $k_{\text{off}}^R = 5\times10^{-3}$ $[s^{-1}]$ (mean interaction time ≈ 200 seconds). This coarse-grained model is sufficient to account for all of our experimental observations and reveals that the release rate of IFNγ from the cell must be extraordinarily slow.

0.1.5 mRNA transcription

We begin our model of mRNA transcription with the central dogma of molecular biology: briefly, DNA is transcribed into mRNA at some rate $k_{\text{transcription}}$, and the mRNA chemically degrades over time with a rate k_{decay}. This is summarized in the simple equation:

$$\frac{d\,\text{mRNA}}{dt} = k_{\text{transcription}}(t) - \text{mRNA}\cdot k_{\text{decay}}^{\text{mRNA}}. \tag{6}$$

We want to explore how exposure to IFNγ affects transcription of mRNA. As described in section 0.1.8, by exposing cells to a constant concentration of IFNγ, we measure how the rate of mRNA transcription changes with time.

$$k_{\text{transcription}}(t) = \frac{d\,\text{mRNA}}{dt} + \text{mRNA} \cdot k_{\text{decay}}^{\text{mRNA}}. \tag{7}$$

We observe an adaptation period where transcription increases before reaching a new elevated constant rate (Figure S4B). These dynamics can be approximated by an exponential approach with timescale $\tau_{\text{adaptation}} = \frac{1}{\beta_{\text{adaptation}}} \approx 12h$ (Figure S4C). Moreover, the new, elevated, steady state, is pSTAT1 dependent, with higher levels of pSTAT1 translating to a higher level of transcription (data not shown). In our model of the system, pSTAT1 is taken to linearly increase the maximal rate of transcription: $k_{\text{transcription}} \rightarrow k_{\text{transcription}}^{\text{basal}} + \alpha_p \left(1 - \exp\left(-\beta_{\text{adaptation}} t\right)\right) \text{pSTAT1}$, where the constant α_p represents an arbitrary proportionality between increase in relative transcription and pSTAT1. $k_{\text{transcription}}^{\text{basal}}$ is the basal rate of transcription, in the absence of IFNγ stimulus. The updated transcription equation (6) is now:

$$\frac{d\,\text{mRNA}}{dt} = k_{\text{transcription}}^{\text{basal}} + \alpha_p \left(1 - \exp\left(-\beta_{\text{adaptation}} \cdot t\right)\right) \text{pSTAT1} - \text{mRNA} \cdot k_{\text{decay}}^{\text{mRNA}}. \tag{8}$$

Since the absolute levels of mRNA are cumbersome to determine experimentally, and our interest is only in the IFNγ induced fraction of the transcripts, we can disregard the steady-state fraction of the equation:

$$\frac{d\,\text{mRNA}_{\text{IFN}\gamma}}{dt} = \alpha_p \left(1 - \exp\left(-\beta_{\text{adaptation}} \cdot t\right)\right) \text{pSTAT1} - \text{mRNA}_{\text{IFN}\gamma} \cdot k_{\text{decay}}^{\text{mRNA}}. \tag{9}$$

0.1.6 Dynamics of mRNA transcription with IFNγ catch-and-release

Next, we incorporate our model for pSTAT1 dynamics (Eq. 5, Figure 4E-F) into our model of mRNA transcription (Eq. 9) and generate the dynamics of mRNA in the days subsequent to IFNγ exposure:

$$\frac{d\,\text{mRNA}_{\text{IFN}\gamma}}{dt} = \alpha_p \left(1 - \exp\left(-\beta_{\text{adaptation}} \cdot t\right)\right) \text{pSTAT1}(t) - \text{mRNA}_{\text{IFN}\gamma} \cdot k_{\text{decay}}^{\text{mRNA}}. \tag{10}$$

Given the dynamics of pSTAT1, our model predicts a rise in the level of mRNA that persists for 2 days following the initial exposure to cytokine. mRNA levels then begin to decrease and return to their original baseline levels around 7 days past-exposure. This is consistent with our experimental measurements of IFNγ regulated mRNA transcripts in the system (Figure 1E, 4F).

Finally, we use the mRNA dynamics to calculate protein:

$$\frac{d\,\text{protein}_{\text{IFN}\gamma}}{dt} = k_{\text{translation}} \cdot \text{protein}_{\text{IFN}\gamma} - k_{\text{decay}}^{\text{protein}} \cdot \text{protein}. \tag{11}$$

0.1.7 Model Parameters

Parameter	Value	Unit	Source
EC_{50}	2.7 ± 1	pM	Our experiments, Figure S4A
k_{catch}^{X}	$1.2 \pm 0.1 \cdot 10^{5}$	$M^{-1}s^{-1}$	Our experiments, Inferred from Figures 4B,D
k_{release}^{X}	$3.7 \pm 0.4 \cdot 10^{-5}$	s^{-1}	Our experiments, Figure 4D
k_{removal}	24	h^{-1}	Our experiments (data not shown)
$k_{\text{decay}}^{\text{mRNA}}$	$2.9 \cdot 10^{-2}$	h^{-1}	Schwanhausser et al., 2011
$k_{\text{decay}}^{\text{protein}}$	$2.3 \cdot 10^{-1}$	h^{-1}	Schwanhausser et al., 2011
$k_{\text{translation}}$	0.1	$\text{mRNA}^{-1} \cdot s^{-1}$	Schwanhausser et al., 2011
$\beta_{\text{adaptation}}$	$8 \pm 3 \cdot 10^{-2}$	h^{-1}	Our experiments, Figures S4B,C
k_{on}^{R}	$7.3 \cdot 10^{6}$	$M^{-1}s^{-1}$	Sadir et al., 1998
k_{off}^{R}	$5 \cdot 10^{-3}$	s^{-1}	Sadir et al., 1998
IFNγR	$2 \cdot 10^{3}$	molecules$\cdot$cell^{-1}	Cofano et al., 1996
X	4700 ± 800	molecules$\cdot$cell^{-1}	Fit in this study
$k_{\text{on}}^{\text{HS}}$	$3.5 \cdot 10^{5}$	$M^{-1}s^{-1}$	Salek-Ardekani et al., 2000
$k_{\text{off}}^{\text{HS}}$	$1.9 \cdot 10^{-2}$	s^{-1}	Salek-Ardekani et al., 2000

Table 1: Parameters used to model the dynamics of our system.

0.1.8 Determining transcriptional adaptation time

3.5×10^4 B16 cells were seeded per well of a 96-well plate and stimulated with a constant dose of 10nM IFNγ. RNA was harvested periodically, cDNA prepared, and the kinetics of *h2kb* accumulation were quantified by RT-qPCR. From these data, we computed the the transcription rate using (see section 0.1.5):

$$k_{\text{transcription}}(t) = \frac{d\,\text{mRNA}}{dt} + \text{mRNA} \cdot k_{\text{decay}}^{\text{mRNA}}. \tag{12}$$

We reasoned that by exposing cells to a constant, saturating concentration of IFNγ, we could assess how the transcription rate changes with time. The transcription rate data were then fitted with a single exponential approach curve. We denote this timescale as: $\tau_{\text{adaptation}} = \frac{1}{\beta_{\text{adaptation}}} \approx 12h$.