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Emergence of macroscopic simplicity from the Tumor Necrosis Factor-alpha signaling dynamics

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Abstract–The Tumor Necrosis Factor-α (TNF-α), a cytokine produced during the innate immune response to invading pathogens, is involved in numerous fundamental cellular processes. Here, to understand the temporal activation profiles of TNF-α regulated signaling network, we developed a dynamic computational model based on perturbation-response approach and the law of information (signaling flux) conservation. Our simulations show that the temporal average population response of the TNF-α stimulated transcription factors NF-κB and AP-1, and 3 groups of 180 downstream gene expressions follow first-order equations. Using the model, in contrast to a well-known previous study, our model suggests that the continuous activation of the third group of genes is not mainly due to the poor rate of mRNA decay process, rather, the law of signaling flux conservation stipulates the presence of secondary signaling, such as feedback mechanism or autocrine signaling, is crucial. Although the living system is perceived as sophisticated and complex, notably, our work reveals the presence of simple governing principles in cell population dynamics.

1. Introduction

The TNF-α is a major proinflammatory cytokine that regulates myriad critical cellular processes such as cell proliferation, differentiation, growth and programmed cell death or apoptosis. Although TNF-α circulates in the mammalian system at baseline levels, its concentration becomes elevated on recognition of invading pathogens by the immune cells or in the proinflammatory disease state [1]. Originally discovered in 1975, the TNF-α binds to the outer membrane bound receptors on target cells and induces a sequence of intracellular molecular reactions that regulates the activities of key survival and proinflammatory transcription factors (TFs) NF-κB and AP-1. This subsequently aids in the elimination of foreign intrusion by triggering the adaptive immunity.

Due to its ability to signal numerous cellular processes, the TNF-α signaling research has received immense attention in recent years, especially on understanding the downstream signaling cascades to regulate and control in response to treat proinflammatory diseases and cancer. Despite numerous studies, we are still at an infant stage in respect to disease treatment. More recent works have generated high throughput time-course data of intracellular signaling molecules. However, without appropriate theoretical and computational tools, it is a huge challenge to interpret the vital data. In this paper, we analyzed the TNF-α signaling dynamics of fibroblast cells using a systemic approach of combining computational technique with experimental data.

Previously, we have used the perturbation–response approach to investigate the well-characterized innate immune response to invading pathogen based on the Toll-like receptor signaling pathways. By using small perturbation approximation resulting in first-order response equations, we developed response rules that were used to infer novel signaling features such as unknown intermediates, crosstalk mechanisms [2,3], and introduced the concept of *signaling flux redistribution* (*SFR*), based on the law of information conservation, where the entire alternative pathway is enhanced when molecules at pathway junctions are removed [4].

Here, we adopted a similar approach to the study of the TNF-α signaling. Starting from a literature curated signaling topology, we developed a dynamic computational model and determined the parameter values by fitting model with time-course experimental data of NF-κB and AP-1 in wildtype, and analyzed the resultant model in different mutant conditions. Overall, our work sheds light into the presence of simple linear response governing the complex innate immune response based on the TNF-α signaling.

2. Materials and methods

To briefly discuss, a fixed perturbation of the input species (e.g. TNF-α) generates downstream response waves of *n* output species (e.g. NF-κB and AP-1) from the reference steady-state that is conserved in terms of information propagation. In general, the resultant changes in species concentrations are governed by the kinetic evolution equation:

$$\frac{\partial X_i}{\partial t} = F_i(X_i, X_2, \dots, X_n), i = 1, \dots, n \qquad (1)$$

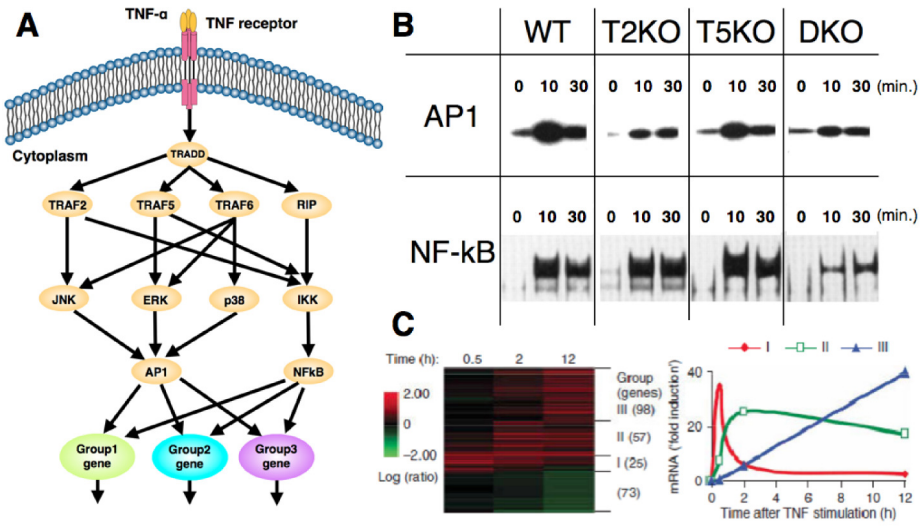

Figure 1. TNF-α signaling pathway and experimental data. A) Schematic view of the TNF-α signaling pathway. **B)** Experimental profiles of AP-1 and NF-κB in each condition (WT, TRAF2KO, TRAF5KO and TRAF2/5 double KO)[5]. **C)** TNF-activated genes can be categorized into three different groups. Left panel: microarray analysis of gene expression in 3T3 fibroblasts stimulated for 0.5, 2 or 12 h with recombinant mouse TNF. Red indicates activated genes, green indicates genes suppressed by TNF treatment. Numbers in parentheses (right margin) indicate the number of genes in each group. Right panels: expression profiles of genes in *groups I, II* and *III* [6].

where the corresponding vector form of Eq. 1 is $\frac{\partial \boldsymbol{X}}{\partial t} = \boldsymbol{F}(X)$ and $\boldsymbol{F}$ is a vector of any non-linear function including diffusion and reaction of the species vector $\boldsymbol{X} = (X_1, X_2, .., X_n)$, which represents activated levels of signaling molecules, for example, through phosphorylation and binding concentration of transcription factors to promoter regions. The response to perturbation can be written by $\boldsymbol{X} = \boldsymbol{X}_0 + \delta\boldsymbol{X}$, where $\boldsymbol{X}_0$ is the reference steady-state vector and $\delta\boldsymbol{X}$ is the relative response from steady-states ($\delta\boldsymbol{X}_{t=0} = 0$).

For small perturbation around steady-state, the higher-order terms in Eq. 1 become negligible, resulting in the approximation of the first-order term. In vector form $\frac{d\delta\boldsymbol{X}}{dt} \approx \left.\frac{\partial \boldsymbol{F}(X)}{\partial \boldsymbol{X}}\right|_{\boldsymbol{X}=\boldsymbol{X}_0} \delta\boldsymbol{X}$, where the zeroth order term $\boldsymbol{F}(\boldsymbol{X}_0) = 0$ at the steady-state $\boldsymbol{X}_0$ and the Jacobian matrix or linear stability matrix is $J = \left.\frac{\partial \boldsymbol{F}(X)}{\partial \boldsymbol{X}}\right|_{\boldsymbol{X}=\boldsymbol{X}_0}$. The elements of J are chosen by fitting $\delta\boldsymbol{X}$ with corresponding experimental profiles and the activation topology. Note that Jacobian matrix elements (response coefficients) can include not only reaction information, but also spatial information such as diffusion and transport mechanisms. Hence, the amount of response (flux propagated) along a signaling pathway can be determined using first order mass-action response, $\frac{d\delta\boldsymbol{X}}{dt} = J\delta\boldsymbol{X}$. This is valid especially for average cell response investigated for time points with a restricted range, usually before 120 min, where post-transcriptional and translational regulations (e.g. feedback or feedforward mechanisms) are insignificant [3,7].

The amount of fixed perturbation chosen for the model depends on the experimentally stimulated concentration of TNF-α, while the parameter values of the downstream species used were chosen to fit temporal experimental NF-κB and AP-1 activation in wildtype condition as training set. The validity of each parameter set was tested on three different knockout (KO) conditions namely, TRAF2 KO, TRAF5 KO and TRAF2/TRAF5 double KO (DKO) (Fig. 1B), to finalize a robust TNF-α model.

We next extended the model to simulate the profiles of 3 distinct groups of gene expressions (Fig. 1C). The 3 groups where identified recently from 180 upregulated genes by Shengli *et al.* [6]. In group *I*, the time of peak in activation is early (~0.5h) and decay is fast. In group *II*, the peak in activation is delayed (~2h) and the decay is slow. In group *III*, the peak in activation occurs after 12h and decay rate is very low compared to transcription rate (Fig. 1C).

3. Results

3.1 Simulations of NF-κB and AP-1 display linear response

Upon recognition of TNF-α, the TNF receptor recruits TRADD to its intracellular domain to activate TRAF-2, -5, -6 and RIP1. These molecules subsequently activate NF-κB and AP-1 through IKK and MAP kinases respectively (Fig. 1A). We used Eq.1 to represent each reaction and chose each parameter value to fit the semi-quantitative data of experimental NF-κB and AP-1 activations (Fig. 1B and 2A). Due to the lack of experimental data for most known molecules in TNF-α signaling, there is a possibility that several sets of parameter values could reproduce the wildtype NF-κB and AP-1 data. Thus, to prevent biased selection process, we tested each set of parameter values in other available

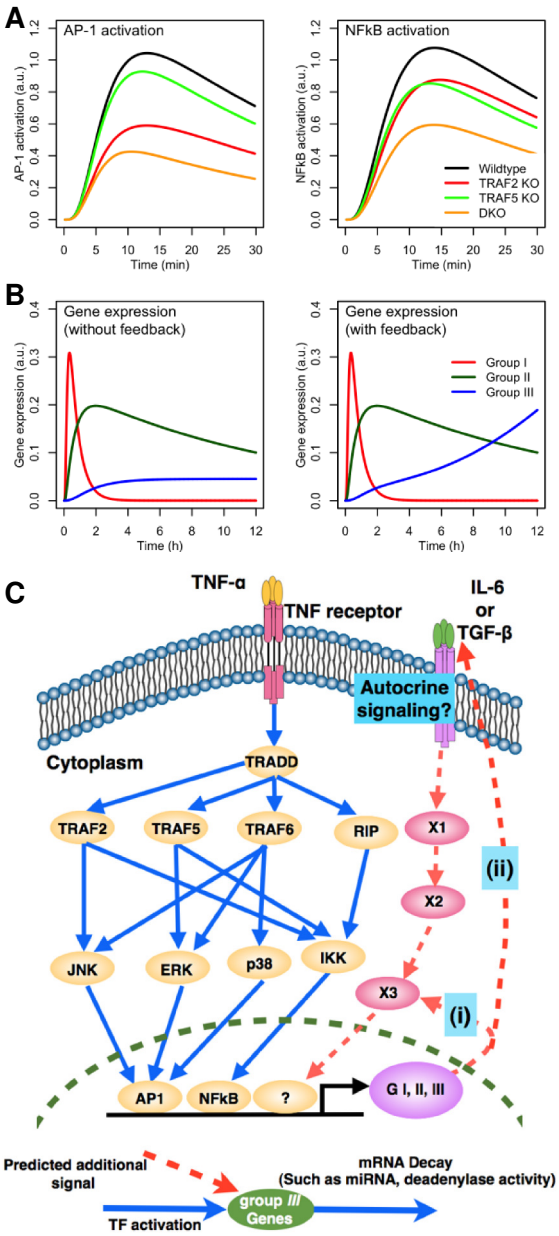

Figure 2. Simulation results and the prediction of novel feedback mechanisms. A) Time course simulation results of AP-1 and NF-kB fitted to experimental temporal profiles. **B)** Left panel: simulation profiles of the 3 group of genes before adding extra feedback signal. Right panel: simulation results after adding the extra feedback signal for group *III* after 2h. **C)** Proposed network topology to fit experimental profiles of all groups of genes, bottom panel represents a schematic of the activation mechanism of group *III* genes by both primary signaling induced by TNF-α (blue arrow) and (i) post-translational feedback or (ii) autocrine signaling (dotted red arrow).

conditions, namely TRAF2 KO, TRAF5 KO and TRAF2/TRAF5 double KO (DKO).

We identified one set of parameters that is successfully able to simulate all 4 experimental conditions with good accuracy. That is, both NF-κB and AP-1 activations reach peak around 10 min and gradually decays at 30 min and that the WT produces the highest activation and the DKO the least (Fig. 2A). This result alone suggests that the dynamics of key TFs NF-κB and AP-1 follows linear response after TNF-α stimulation, at least for the first 30 min of investigation.

3.2 Analyzing gene expression patterns reveals novel transcription process

From the analysis of 180 upregulated genes, recent works have identified 3 distinct gene groups, with possibly corresponding distinct biological roles [6]. The mechanistic reasons for the distinct temporal profiles have been attributed mainly due to the differential regulation of the decay process of the gene (mRNA) expressions by miRNA targeting the AU Rich Element (ARE) region on the 3'UTR. The lesser ARE regions (causing lower decay) for group *III* genes, compared with groups *I* and *II*, have been suggested for its continuous activation profile (Fig. 1C).

Here, we wondered whether there are alternative explanations for the distinct gene expressions, especially focusing on the role of not only decay but also transcription process. In our approach, the decay process can be represented by a depletion or deactivation term. That is, the overall decay term collectively represents mRNA instabilities, deadenylase and miRNA regulations. The formation term is represented by the transcription process.

Thus, we extended the model to simulate downstream gene expression profiles. Notably, using linear response, we were able to successful fit group *I* and *II* genes, but not for group *III* genes (Fig. 2B, left). According to our model, reducing the decay term alone is not able to produce continuous activation of group *III* genes. Rather, this leads to an increase followed by a stabilization of the profile. However, the addition of new feedback transcription process is able to overcome the limitation of model simulation (Fig. 2B, right and 2C).

3.3 Predicting group *I*, *II* & *III* gene expressions in multiple KOs

TNF-α signaling is enhanced in proinflammatory diseases and cancer [1]. Next, using our revised model, we simulated, *in silico*, the effects of TRAF2 KO, TRAF5 KO, TRAF6 KO and RIP1 KO for group *I*, *II* and *III* genes (Fig. 3). Overall, the simulations suggest that TRAF2 is a key single target molecule to control the proinflammatory response to TNF-α stimulation.

4. Discussion

In this paper, we investigated the temporal activation dynamics of transcription factors NF-κB, AP-1 and 3 groups of genes representing 180 upregulated genes in TNF-α stimulation. Our model developed using first-order response equations was sufficient to simulate the profiles

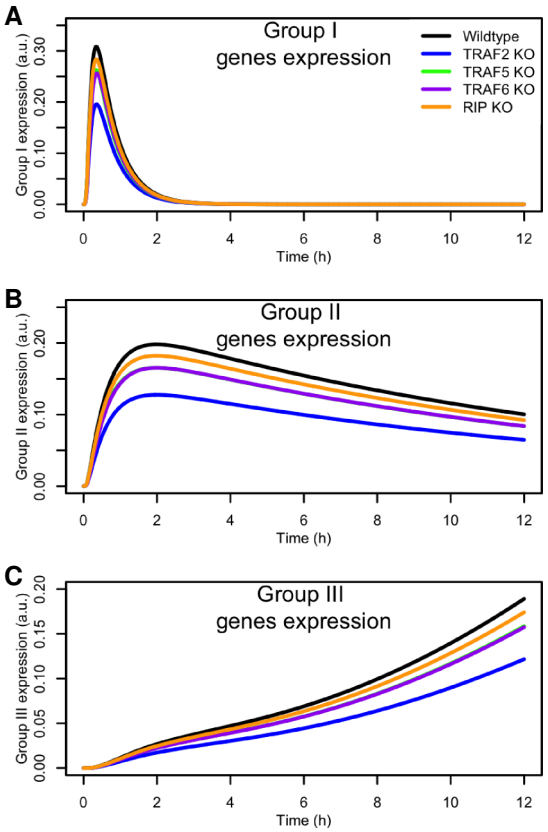

Figure 3. ***In silico*** **KO effects on the 3 groups of genes.** Simulation profiles of the 3 group of genes A) group *I*, B) group *II*, C) group *III*, in wildtype and 4 KO conditions (TRAF2, TRAF5, TRAF6 and RIP1 KO). Note that TRAF5 KO (green curve) is hidden by TRAF6 KO (purple).

of NF-κB and AP-1 in wildtype, TRAF2 KO, TRAF5 KO and TRAF2/TRAF5 double KO (DKO). This result is surprising has it is envisioned that the innate immune response of TNF-α is highly complex.

Next, analyzing the downstream gene expression profiles, our model suggests that the continuous activation of group *III* genes is not due to lesser ARE region alone resulting in lower decay process, but also due to additional novel transcription process. This could either be provided by secondary signaling features such as autocrine signaling through IL-6 and TGF-β, or derives from post-transcriptional feedback mechanisms regulating the novel promoter regions of group *III* genes (Fig. 2C). Notably, recent works have highlighted the role of interferon (IRF) transcription factor family, in *Ccl5* [8], which belong to one of the group *III* genes. Further experimental work is required to elucidate the exact mechanisms.

Finally, to find a way to regulate TNF-α signaling, which is enhanced in proinflammatory disease and cancer, we simulated the profiles of the 3 group of genes by simulating the *in silico* KO effects of TRAF2 KO, TRAF5 KO, TRAF6 KO and RIP1 KO. We find that TRAF2 KO is the best candidate to regulate the levels of proinflammatory molecules. In summary, our work highlights the existence of simple governing principles in complex innate immune process which is useful to elucidate crucial novel signaling features.

Acknowledgments

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