Investigation of Negative Feedback in Interleukin-17 Receptor Signaling

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We present a computational model of signaling downstream of the IL-17 receptor. This model encompasses major signaling components that activate NF-κB and in turn promote the production of pro-inflammatory cytokines. NF-κB also promotes production of A20, a deubiquitinating enzyme. We recently showed that A20 acts as a negative regulator of the IL-17 pathway. By modeling A20 interactions with TRAF6 and IKK, we recapitulate experimentally-observed oscillations in NF-κB–dependent A20 expression at both the mRNA and protein levels. Expansion of the model, along with additional experimental work, will enable us to explore novel hypotheses about regulatory mechanisms in IL-17 signaling.

I. Background

IL-17 is a recently discovered cytokine produced by CD4+ Th17 cells in response to infection that plays a critical role in the immune response to extracellular pathogens, particularly fungal infections, while also being connected to autoimmune disorders such as psoriasis and rheumatoid arthritis [1]. It functions by signaling through a heterodimeric receptor, beginning a signaling cascade that results in the activation of the transcription factor NF-κB. This leads to the production of pro-inflammatory cytokines such as TNF-α and IL-1β, as well as anti-inflammatory mediators such as A20 [2]. A20 acts as a negative feedback regulator on IL-17 signaling, inhibiting further activation of NF-κB [3]. Although certain inhibitory mechanisms of A20, such as the deubiquitination of TRAF6, are known others remain unclear [4]. Further study of the IL-17 signaling pathway is required to fully understand the details of how the cell processes the signal, and how regulators like A20 modulate this signal. Mathematical modeling gives us a systematic way to explore proposed negative feedback mechanisms of A20 as well as additional candidate inhibitors.

II. Results

A. Detailed model of IL-17 signaling

Our model considers stimulation by varying doses of IL-17a. Signaling is processed through numerous signaling intermediates, some unique to the IL-17 pathway while others are shared between multiple pathways. This results in the activation of NF-κB and the transcription and translation of selected target genes. We model this complex signaling system using the rule-based modeling approach [5], in which signaling proteins are modeled as structured objects and rules describe their biochemical interactions. The model contains 19 molecules and 46 reaction rules that expand to a reaction network with 168 distinct chemical species and 5,145 unidirectional reactions, which we simulate using ODEs.

B. Experiments and parameter estimation

We have collected experimental data showing the dynamics of A20 transcript and protein levels following IL-17 stimulation. Additionally, data from the literature shows the early dynamics of adaptor Act1 binding IL-17R and TRAF6 ubiquitination [6]. This data was incorporated into our model using Bayesian parameter estimation [7] augmented by parallel tempering [8], which allowed us to efficiently sample the large space of kinetic parameters. The parameterized model quantitatively recapitulates our time course data for A20, including peaks at 30 minutes and 6 hours post-stimulation, which exhibits damped oscillations similar to those seen in other NF-κB-driven systems [9]. The model also captures transient binding between the IL-17 receptor and the adaptor Act, peaking at 15 minutes.

C. Negative feedback mechanisms of A20

Incorporating recently-discovered A20-mediated negative regulation of TRAF6 activation [3] allows the model to recover important negative feedback features. Knocking out A20 in simulated experiments results in sustained levels of NF-κB activation, replacing the oscillations seen in wild type simulations, a prediction we hope to confirm experimentally. Additionally, our model will allow us to probe the validity of additional candidate A20 regulatory mechanisms in the IL-17 pathway, such as the deubiquitination of IKK, as observed in TNFR signaling [10].

III. Conclusion

Our model accurately captures the observed dynamics of a number of signaling intermediates in the IL-17 pathway. The model makes distinct predictions for various potential negative feedback mechanisms involving A20, which we are currently in the process of testing experimentally.

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References

[1] Hueber, W. et al. *Sci Transl Med* **2**, 52-72 (2010)
[2] Aggarwal, S. and Gurney, A. *J Leukoc Biol*. **71**, 1-8 (2002).
[3] Garg, A. et al. *Sci Signal* **6**, ra44 (2013)
[4] Heyninck, K. and Beyaert, R. *FEBS Lett* **442**, 147-150 (1999).
[5] Faeder, J. R., Blinov, M. L. & Hlavacek, W. S. *Methods Mol. Biol* **500**, 113–167 (2009).
[6] Liu, C. et al. *Sci Signal* **2**, ra63 (2009)
[7] Eydgahi, H. et al. *Mol Sys Biol* **9**, 644 (2013).
[8] Earl, D. J. and Deem, M. W. *Phy Chem Chem Phys* **7**, 3910-3916 (2005).
[9] Nelson, D. E. et al. *Science* **306**, 704-708 (2004).
[10] Mauro, C. et al. *J Biol Chem* **281**, 18482-18488 (2006)