Cellular Regulation of NF-kB

Group 2
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I. CONTRIBUTIONS

1.1 Presentation

Brandon Dai: Created Linear Model. Presented Steady State Analysis, Linear Model, and Physiological System slides.
Chelsea Lang: Worked on and presented NF-kB Signaling Pathway, System Components, and Variables slides. Also worked on Block Diagram, Assumptions, Disturbance Characterization.
Joey Soliman: Worked on and presented Block Diagram, Literature, and Assumptions slides. Also worked on Steady State Analysis and Disturbance Characterization.
Ramzi Tweini: Presented Mathematical Relationship of Block Diagram Components and Disturbance slides.

1.2 Final Report

Brandon Dai: Block Diagram, Linear Model, Conclusion
Chelsea Lang: Background and Significance; System Properties, Variables, and Assumptions; Block Diagram
Joey Soliman: Disturbance Characterization/Laplace Transformation, Literature Values, Steady State Analysis, Mathematical Relationship of Block Diagram Components
Ramzi Tweini: Block Diagram, Steady State Analysis

II. BACKGROUND AND SIGNIFICANCE

The NF-kB signaling pathway is important for regulating processes of immune and inflammatory responses. NF-kB acts as a transcription factor and induces the expression of various pro-inflammatory genes, including those encoding cytokines and chemokines. Misregulation of NF-kB activity has been identified as a major culprit of chronic inflammatory diseases and cancer.[4] Within the NF-kB signaling pathway, there is also a canonical and non-canonical pathway. The canonical pathway relies on the inducible degradation of IKBs leading to translocation of the p50/RelA dimer into the nucleus, while the non-canonical pathway activates RelB/p52 NF-kB complex.[5] They differ not only in the cell receptors and adaptors, but also in the signaling mechanisms. Within these pathways, there is also a positive and negative feedback loop that occurs. The positive feedback of the system stems from the active form of NF-kB, which is translocated into the nucleus to induce transcription of itself and apoptotic genes. The NF-kB activity will continue to increase until apoptosis finally occurs. The negative feedback of the system stems from the inactive form of NF-kB. In this case, the homeostatic regulation of NF-kB from entering the nucleus allows NF-kB to remain inactive in the cytoplasm. Therefore, the negative feedback system ultimately keeps NF-kB from entering the nucleus to keep the cell alive. This report will focus on the mechanisms by which the canonical NF-kB signaling pathway is regulated through negative feedback.

The pathway begins in the normal state with an inactive form of NF-kB. When the TNF signal binds to the TNF receptor, the signaling pathway is triggered leading to activation of IKK, an IkB kinase, which phosphorylates the IkB protein. After phosphorylation of the IkB protein, it gets ubiquitinated, also

referred to as proteasome degradation. Because IkB is no longer inhibiting NF-kB, NF-kB becomes active and enters the nucleus. Acting as a transcription factor, NF-kB binds to the promoters on DNA and expresses itself and apoptotic genes. Once a certain threshold of NF-kB is reached in the nucleus, cell apoptosis occurs.

III. SYSTEM PROPERTIES, VARIABLES, AND ASSUMPTIONS

3.1 Properties

The components of the NF-kB signaling pathway that are considered include the nucleus, the entire B cell, and the post-translational modification proteins. The post-translational modification proteins are the effectors, which include IKK phosphorylation and IkB ubiquitination or proteasome degrade. The nucleus is the control center and sensor, because it sends a feedback signal back to itself to increase or decrease post-translational modifications based on levels of NF-kB activity. Other system properties include the activators and inhibitors. The activators for the pathway are the IKK proteins because they phosphorylate the IkB proteins. The TNF receptor is another activator because after the signal binds to the receptor, IKK is activated. The inhibitors are the IkB proteins because they keep NF-kB inactive in the cytoplasm.

3.2 Variables

The variables included in the negative feedback system include input and output of homeostasis and NF-kB activity in the nucleus, respectively. In this case, the input would be the nominal levels of NF-kB in the nucleus. Because our system is regulating NF-kB from entering the nucleus, the nominal level would be 0. The output is the levels of NF-kB activity in the nucleus.

3.3 Assumptions

Assumptions are made in order to explain system properties and variables and ultimately create our model of the pathway. The first assumption is that only specific activators and inhibitors are considered. In our model, NF-kB activity is assumed to only be affected by IKK and IkB proteins. The second assumption is that the system is affected by an immediate response to the stimuli, where the pathway occurs after one signal binds the specific receptor. The third assumption is that the NF-kB dimer complex is broken into the specific dimers that induce apoptotic gene expression. The last assumption is that our system is only affected by the negative feed system of the canonical pathway. In the real physiological system, many signals and receptors contribute to the system. The canonical and non-canonical pathway and negative and positive feedback also occur simultaneously to affect NF-kB levels in the nucleus.

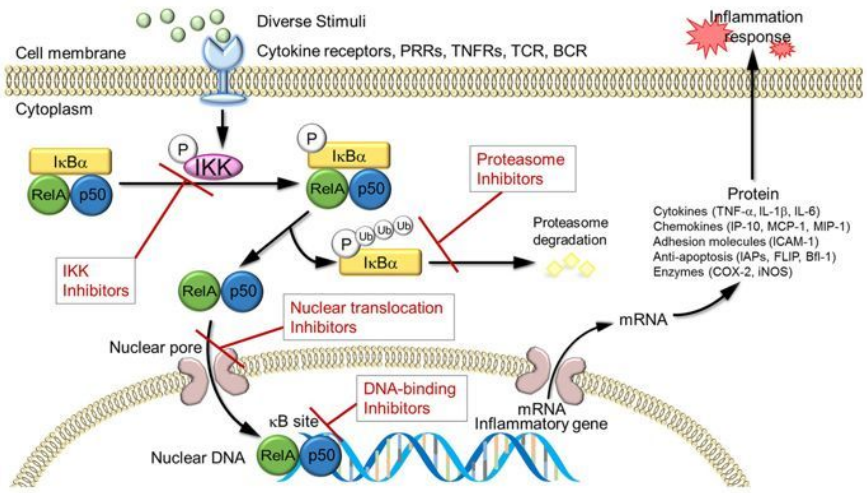

Figure 1. NF-kB Signaling Pathway[3]

IV. BLOCK DIAGRAM

4.1 Block Diagram

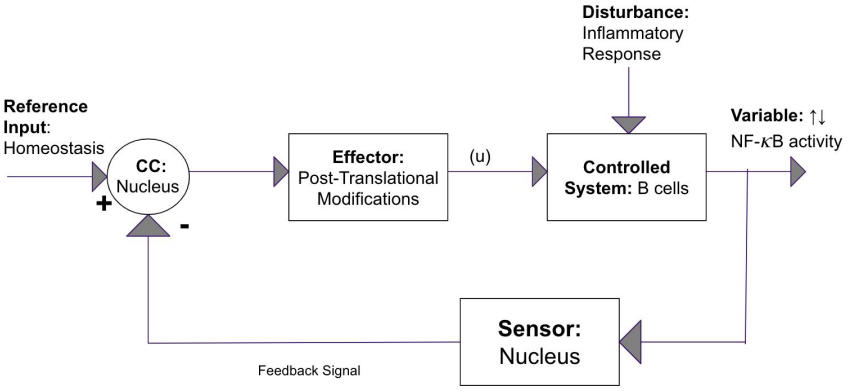

Figure 2. Block Diagram of Cellular Regulation of NF-kB

In our model of the cellular regulation of NF-kB, the control center and sensor are the nucleus. The effectors are the post-translational modification proteins and the effector actions include IKK phosphorylation and IkB ubiquitination or proteasome degradation. The inflammatory response, or when the TNF ligand binds to the TNF receptor on the cell membrane, represents the disturbance in the system affecting the controlled system, which is the entire cell. As the sensor, the nucleus detects the disturbance and will signal to itself as the control center to increase or decrease the number of post-translational modification proteins in order to keep NF-kB in the cytoplasm.

V. MATHEMATICAL ANALYSIS

5.1 Steady State Analysis

The steady state analysis of the negative feedback system of the NF-kB signaling pathway can be applied in two cases. The first case of steady state is at homeostasis, where the NF-kB activity levels are at 0. This would be the case if there is no disturbance input into the controlled system. The second case of steady state to be considered in our block diagram is when there is a consistent disturbance. If the disturbance is consistent, the response of the controlled system will increase NF-kB activity. NF-kB activity will continue to increase because IKK proteins will continuously be sent out to phosphorylate the IkB complexes. However, the homeostatic response of our negative feedback system will fight back against this increase in activity by decreasing the amount of IKK proteins being sent out of the nucleus, but will never be able to completely negate the response. This will result in achieving a steady state, keeping NF-kB activity levels at some arbitrary value. If there is a case where there is an inflammatory response signal present for a certain amount of time, then the same response as case two will occur until the signal leaves and homeostatic response takes over to bring NF-kB levels back to 0, similar to case one.

5.2 Mathematical Relationship of Block Diagram Components

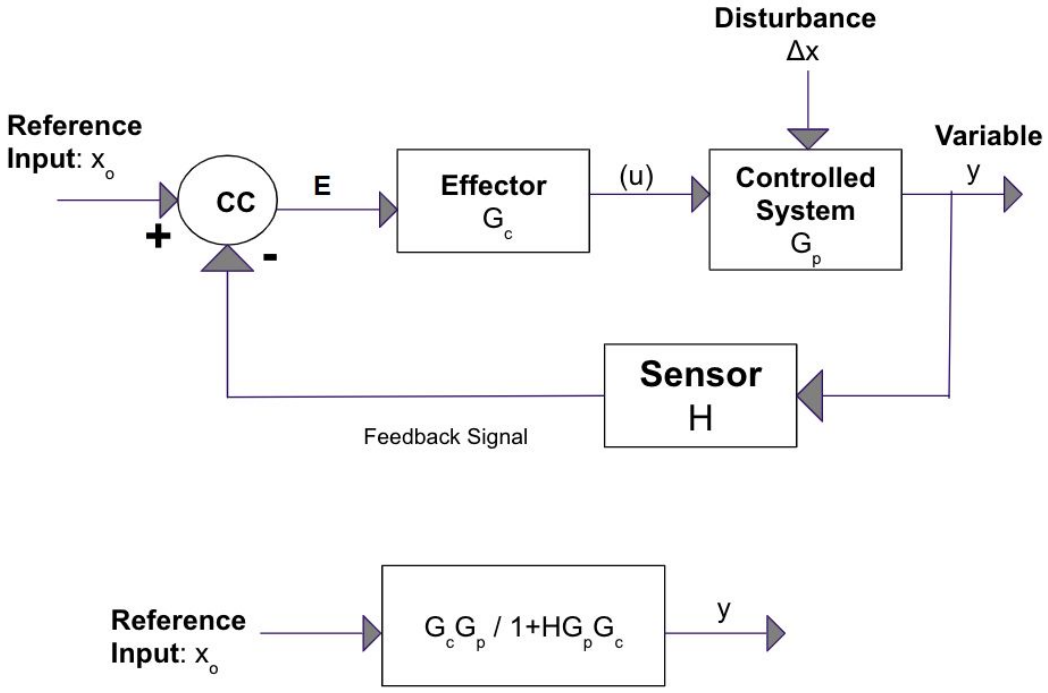

Figure 3. Mathematical Model Relating Block Diagram Components

The block diagram components from Figure 2 are simplified using variables. When simplifying the block diagram to obtain the transfer function, the disturbance is neglected. If we take the block diagram apart further into smaller mathematical equations we get:

$$Y = G_c G_p E$$

$$E = X_0 - HY$$

Plugging in values for $H(1)^1$, $Y(50)^1$, and $X(0)^1$, we get $50 = G_c G_p(0 - 50)$, which further gives us

$$G_c G_p = -1$$

5.3 Disturbance Characterization

The disturbance signal is the inflammatory response, which is when the tumor necrosis factor (TNF) cytokines are released. The TNF is either present or not present at the tumor necrosis factor receptor (TNFR), so we can define our disturbance to be representative of a rectangular pulse function. While TNF is present on the TNFR the whole NF-*k*B signaling pathway will be functioning, but the signal stops as soon as the TNF is removed.

Because the disturbance is characterized as a rectangular function, the Laplace transformation of the rectangular pulse is calculated and shown in Figure 4. When relating the system to the Laplace transformation shown below, the height (h) would be 1 as long as TNF is present, while the time of pulse (t_w) is the time that the TNF is present for.

$$f(t) = \begin{cases} 0 & \text{for } t < 0 \\ h & \text{for } 0 \le t < t_w \\ 0 & \text{for } t \ge t_w \end{cases}$$

$$\mathscr{L}[f(t)] = \int_0^{\infty} f(t)\, e^{-st}\, dt$$

$$= h \int_0^{t_w} e^{-st}\, dt$$

$$= h\,(-1/s)\, e^{-st} \Big|_0^{t_w}$$

$$F(s) = (h/s)\,(1 - e^{-t_w s})$$

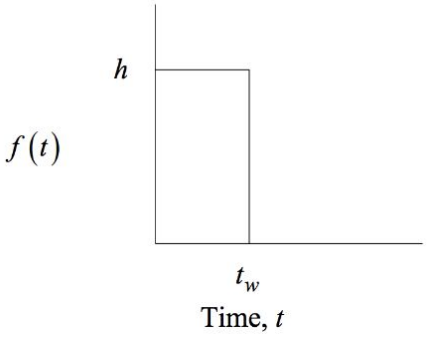

Figure 4. Laplace Transformation

5.4 Literature Values

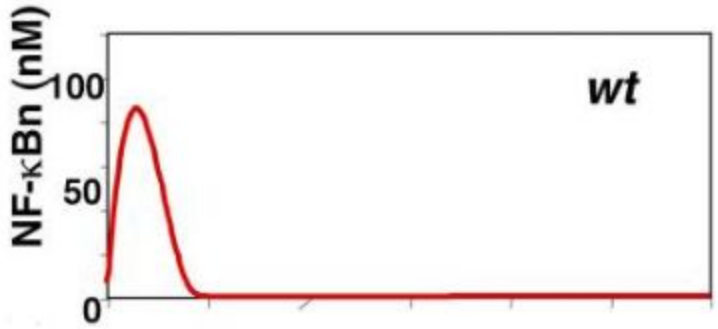

Figure 5. NF-kB response to 15 min TNF exposure[1]

This graph shows the cells response to stimulation by TNF for a period of 15 min, which was obtained from Kearns et al., 2006. Each tick mark on the x-axis is representative of 1 hour. The graph shows us that for the 15 minutes the cell is exposed to TNF, the NF-kB activity continues to increase, but this increase begins to slow down due to the cell's homeostatic response trying to bring the activity levels back down to 0. When the cell is cut off from TNF exposure, the homeostatic response is able to take over completely and bring the activity levels back down to 0. This example is the first case of steady state explained earlier in section 5.1.

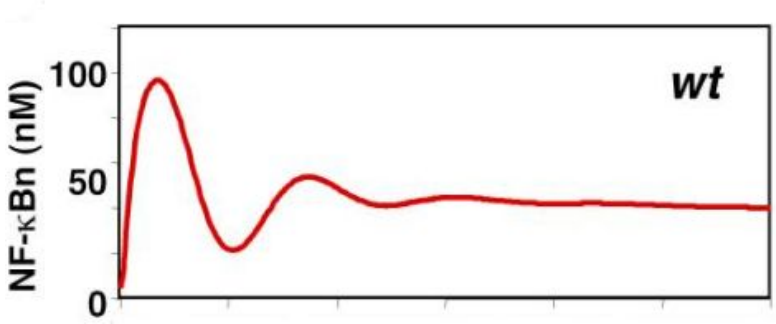

Figure 6. NF-kB response to persistent TNF exposure[1]

This graph shows the cells response to being stimulated by TNF for an indefinite time interval which was also obtained from Kearns et al., 2006. Once again, each tick mark on the x-axis is representative of 1 hour. When comparing this graph to the previous graph in Figure 5, we see that the beginning of the response curve is similar, but the homeostatic response is not enough to completely overpower the NF-*k*B signaling pathway being stimulated by the TNF. The two different responses fight against each other which creates a sinusoidal wave that stabilizes at around 50. Since the disturbance is persistent, the homeostatic response modeled in our block diagram was not able to bring the response back down to 0, but it did bring the system to a steady state with a constant level of NF-kB activity. This is the second case of steady state indicated earlier in section 5.1.

5.5 Linear Model

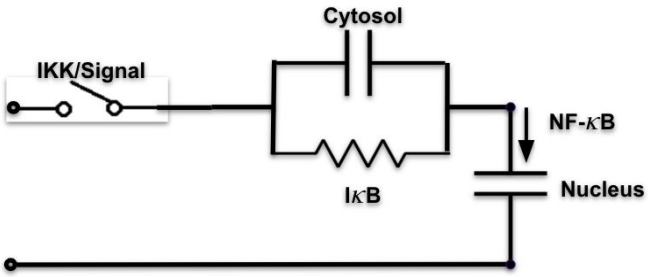

Figure 7. Linear System

The linear model of our system represents the NF-kB pathway as an electronic circuit, rather than its traditional biological representation. To simplify the model, only a fragment of the NF-kB pathway is modeled. The components of the system are chosen because they represent significant effectors in the NF-kB pathway.

For the circuit, the electrical components used are: a switch, capacitors, a resistor, and wire (to carry current). In electrical circuits, switches are used to instantaneously toggle the flow of current throughout the circuit. Capacitors are components that have the ability to store charge flowing through the wires of the circuit. This charge can then be used to transfer to different locations within the circuit. Resistors use the law of conservation of energy to "resist" the current flow in circuits, resulting in a lower current at the output terminal of a resistor.

In our linear model, IKK represents the switch because it is an enzyme-complex that begins the NF-kB pathway and is a prerequisite for the succeeding steps. NF-kB is chosen to represent the wire/current. This is because after NF-kB is activated within the cytosol, it flows to the nucleus. This means the active/inactive form of NF-kB is the source of our potential within the cell. Since NF-kB is defined as the charge and it flows from the cytosol to the nucleus, we can assume that both of these locations are capacitors. The last component in the linear model is the resistor. The resistor is assumed to be IkB because IkB is activated by IKK and IkB regulates NF-kB. For this reason, IkB is assumed to "resist" the flow of NF-kB in our linear model. These adjustments simplify the cellular regulation of NF-kB, allowing for a brief summary of the complex pathway.

VI. CONCLUSION

The NF-kB pathway is an important process by which cells attempt to maintain homeostasis. In our specific analysis of NF-kB, the regulation of apoptosis is our focus. Although this was the primary focal point of this project, the NF-kB pathway also regulates other cellular functions, such as inflammatory responses, cellular proliferation, and maintenance of infected cells.

Selecting the apoptotic regulation of NF-kB allowed simplification of the pathway into a negative feedback loop. With the block diagram created, the system is further simplified by assigning variables to

each component in our block diagram, enabling us to perform a mathematical analysis of the loop by applying various block diagram rules and equivalent circuits. While the mathematical analysis of NF-kB feedback loop gave us a quantitative description of the pathway, a qualitative representation of this pathway is still needed. Our linear model gave us a qualitative relationship between the NF-kB pathway and an electrical circuit. With our mathematical and linear models of the canonical NF-kB pathway, literature values and representations are used to validate the assumptions made in our analysis.

VII. REFERENCES

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