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A MODEL FOR GENE ACTIVATION

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Abstract. The purpose of this paper is to develop a model for the activation of the gene responsible for the production of the cytokine interleukin 6, IL-6. This is motivated by experimental work that indicates that exposure to certain exogenous chemicals results in changes in cytokine production. In particular, exposure to both the widely used pesticide atrazine and the legacy pesticide dieldrin, still very much present in the environment, resulted in the reduction of the production of IL-6. We develop of model of twelve ordinary differential equations to model the effect of changes in transcription factor levels on IL-6 production rates and establish basic qualitative properties of solutions.

1. Introduction

The purpose of this paper is to develop a model for the activation of the gene responsible for the production of the cytokine interleukin 6, IL-6. This is motivated by experimental work that indicates that exposure to certain exogenous chemicals results in changes in cytokine production. In particular, exposure to both the widely used pesticide atrazine and the legacy pesticide dieldrin, still very much present in the environment, resulted in the reduction of the production of IL-6 [5]. We start with a description of the biological problem.

We recall that a cytokine is a signaling protein or glycoprotein used in cellular communication. In particular, IL-6 is important in dealing with immune response to trauma, the bone formation and breakdown cycle, and, perhaps, response to some bacterial attacks [1, 5]. The following surprising result motivated the search for a mathematical model. Doses of dieldrin and Atrazine, which separately produced roughly 10% drops in IL-6 levels, when combined, produced an 80% drop in IL-6 levels, far greater than the expected additive or subadditive effect [5]:

Treatment	IL-6 level post treatment
Dieldrin	0.92
Atrazine	0.88
Both	0.19

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Given that public policy on acceptable exposure levels are based on single exposure measurements, this is bad news.

Further measurements showed that the exposure to the dieldrin and atrazine resulted in the reduction of certain transcription factors within the nucleus of the cell. A transcription factor is a protein that binds to a specific part of the DNA. called a response element. The transcription factors we consider are named $AP-1$, $NF-\kappa B$, and $AP-1-NF-\kappa B$.

The experimental results now look like

Treatment	$AP-1$	$NF-\kappa B$	$AP-1-NF-\kappa B$
Dieldrin	0.7	0.7	0.49
Atrazine	0.65	0.7	0.455
Both	0.35	0.4	0.14

Treatment	IL-6 level post treatment
Dieldrin	0.92
Atrazine	0.88
Both	0.19

The desire now was to model IL-6 production in terms of transcription factors. Thus, we will develop a model that will give the rate of IL-6 production as a function of transcription factor concentration within the nuclei of the cells. To do this we will need to also know the what response elements are important. In the each cell of a mouse there is a gene with two response elements, ARE and NRE. If both response elements are activated by having the appropriate proteins bind to each of them, the gene will start the cell producing IL-6. We have $AP-1$ binding reversibly to the ARE response element. We expect competition for the NRE response element between the $NF-\kappa B$ and the $AP-1-NF-\kappa B$ [3].

In section 2 of this paper, we will produce a model that gives the fraction of response elements filled with a particular transcription factor as a function of time. Section 3 will develop a model showing what fraction of genes are activated and producing IL-6 and how they are activated. Finally, section 4 will give some basic mathematical results for the model developed in section 2.

2. The main physical model

Our initial assumptions are as follows.

1. All genes will be treated as being in a single well mixed solution of cytoplasm.
2. The likelihood of binding to a response element is independent of the status of the other response element.
3. We will only consider three chemical species $AP-1$, $NF-\kappa B$, and $AP-1-NF-\kappa B$.

In the solution, we assume the following reaction is occurring:

$$AP-1+NF-\kappa B \rightleftarrows^{k_1}_{k_2} AP-1-NF-\kappa B$$

and that it is governed by mass action. Using the following variable names

Chemical	Variable
$AP-1$ molarity	c_1
$NF-\kappa B$ molarity	c_2
$AP-1-NF-\kappa B$ molarity	c_3

we obtain the three nonlinear differential equations below

$$\begin{aligned}\frac{dc_1}{dt} &= -k_1c_1c_2 + k_2c_3,\\ \frac{dc_2}{dt} &= -k_1c_1c_2 + k_2c_3,\\ \frac{dc_3}{dt} &= k_1c_1c_2 - k_2c_3.\end{aligned}$$

These equations describe the chemical reactions in solution assuming no other mechanisms are in play.

We now consider the other physical processes in isolation as well. These are $AP-1$ binding reversibly to the ARE response element and competition for binding to the NRE response element between the $NF-\kappa B$ and the $AP-1-NF-\kappa B$.

We assume that we have a total volume V of cytoplasm, our fluid, and that there are M moles of cells. The units for the solution concentrations, c_j, will be molarities, and the sorbed concentrations, q_j, will be in moles per mole.

We model this as we would a sorption process [4].

Chemical	Variable
$AP-1$ molarity	c_1
$NF-\kappa B$ molarity	c_2
$AP-1-NF-\kappa B$ molarity	c_3
Moles of $AP-1$ per mole of ARE	q_1
Moles of $NF-\kappa B$ per mole of NRE	q_2
Moles of $AP-1-NF-\kappa B$ per mole of NRE	q_3

We make the assumption that at equilibrium for a fixed set of solution concentrations we have a fixed fraction of response elements filled:

$$\begin{aligned}q_1 &= f_1(c_1),\\ q_2 &= f_2(c_1, c_3),\\ q_3 &= f_3(c_1, c_3).\end{aligned}$$

Competition for the NRE response element between the $NF-\kappa B$ and the $AP-1-NF-\kappa B$ will mean that f_2 will be increasing in c_2 and decreasing in c_3 and f_3 will be increasing in c_3 and decreasing in c_2. We will assume f_1 is an increasing function and that $f_j = 0$ when $c_j \le 0$ and positive otherwise.

Our initial hope was to use the Langmuir model for a single layer sorption process

$$\begin{aligned}q_1 = f_1(c_1) &= \frac{c_1}{\beta_1 + c_1},\\ q_2 = f_2(c_2, c_3) &= \frac{c_2}{\beta_2 + c_2 + \gamma_3 c_3},\\ q_3 = f_3(c_2, c_3) &= \frac{c_3}{\beta_3 + c_3 + \gamma_2 c_2}.\end{aligned}$$

Unfortunately, experimental measurements of equilibrium data [5] did not conform to our expectations. We will thus use a Langmuir-Freundlich type model that will allow for competition.

$$
\begin{aligned}
q_1 = f_1(c_1) &= \frac{c_1^{\pi_1}}{\beta_1 + c_1^{\pi_1}}, \\
q_2 = f_2(c_2, c_3) &= \frac{c_2^{\pi_2}}{\beta_2 + c_2^{\pi_2} + \gamma_3 c_3^{\pi_{23}}}, \\
q_3 = f_3(c_2, c_3) &= \frac{c_3^{\pi_3}}{\beta_3 + c_3^{\pi_3} + \gamma_2 c_2^{\pi_{32}}}.
\end{aligned}
$$

However, our analysis will not depend on the functional form. That functional form was used to fit data in [5].

We assume that rate of change between sorbed concentration and solution concentration is proportional to the difference from equilibrium:

$$
\begin{aligned}
\frac{dc_1}{dt} &= r_{c1}(q_1 - f_1(c_1)), \\
\frac{dc_2}{dt} &= r_{c2}(q_2 - f_2(c_2, c_3)), \\
\frac{dc_3}{dt} &= r_{c3}(q_3 - f_3(c_2, c_3)), \\
\frac{dq_1}{dt} &= r_{q1}(f_1(c_1) - q_1), \\
\frac{dq_2}{dt} &= r_{q2}(f_2(c_2, c_3) - q_2), \\
\frac{dq_3}{dt} &= r_{q3}(f_3(c_2, c_3) - q_3).
\end{aligned}
$$

We observe that the total number of moles of a given transcription factor is

$$q_j M + c_j V.$$

To maintain conservation of mass, we require

$$r_{qj} = \frac{V}{M} r_{cj}.$$

We will rename

$$r_j = r_{cj}$$

Our full model (so far) is now

$$
\begin{aligned}
\frac{dc_1}{dt} &= r_1(q_1 - f_1(c_1)) - k_1 c_1 c_2 + k_2 c_3, \\
\frac{dc_2}{dt} &= r_2(q_2 - f_2(c_2, c_3)) - k_1 c_1 c_2 + k_2 c_3, \\
\frac{dc_3}{dt} &= r_3(q_3 - f_3(c_2, c_3)) + k_1 c_1 c_2 - k_2 c_3, \\
\frac{dq_1}{dt} &= \frac{V}{M} r_1(f_1(c_1) - q_1), \\
\frac{dq_2}{dt} &= \frac{V}{M} r_2(f_2(c_2, c_3) - q_2), \\
\frac{dq_3}{dt} &= \frac{V}{M} r_3(f_3(c_2, c_3) - q_3).
\end{aligned}
\tag{2.1}
$$

3. The book keeping equations

We will build a probabilistic model on top of the differential equations model which will follow the fraction of cells in each state of activation. We have 6 classes of cells:

Cell type	Moles of cells in class per mole of cells
No bound response sites	p_0
ARE occupied by $AP-1$ but NRE unoccupied	p_1
NRE occupied by $NF-\kappa B$ but ARE unoccupied	p_2
NRE occupied by $NF-\kappa B-AP-1$ but ARE unoccupied	p_3
ARE occupied by $AP-1$ and NRE occupied by $NF-\kappa B$	p_4
ARE occupied by $AP-1$ and NRE occupied by $NF-\kappa B-AP-1$	p_5

We now must use this information to find the values of the p_j as functions of time. We will assume that only one change occurs at a time. That is a single site may become occupied or unoccupied at a time. The diagram below indicates how the states connect, where the edges indicate reversible transformations.

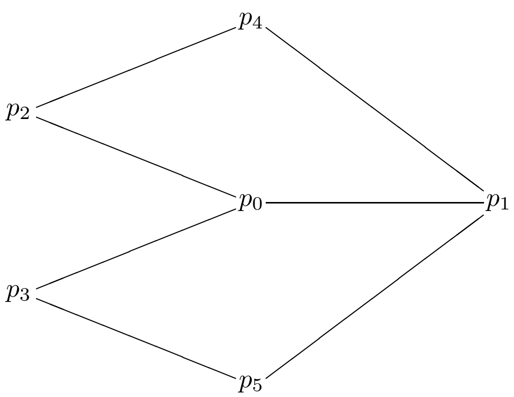

Figure 1. Graph of the activation states of genes

Our model is probabilistic in the sense that the rate of change from one state to another depends on the proportion of cells in the given state at a given time. We will use the notation

$$t^+ = \max(0,t), \quad t^- = (-t)^+.$$

The charts below will indicate how each state is changing, what state it is changing to, and at what rate.

	Transition rate for p_0
Into p_1	$\left(\frac{dq_1}{dt}\right)^+ \frac{p_0}{p_0+p_2+p_3}$
Into p_2	$\left(\frac{dq_2}{dt}\right)^+ \frac{p_0}{p_0+p_1}$
Into p_3	$\left(\frac{dq_3}{dt}\right)^+ \frac{p_0}{p_0+p_1}$

	Transition rate for p_1
Into p_0	$\left(\frac{dq_1}{dt}\right)^- \frac{p_1}{p_1+p_4+p_5}$
Into p_4	$\left(\frac{dq_2}{dt}\right)^+ \frac{p_1}{p_0+p_1}$
Into p_5	$\left(\frac{dq_3}{dt}\right)^+ \frac{p_1}{p_0+p_1}$

	Transition rate for p_2
Into p_0	$\left(\frac{dq_2}{dt}\right)^- \frac{p_2}{p_2+p_4}$
Into p_4	$\left(\frac{dq_1}{dt}\right)^+ \frac{p_2}{p_0+p_2+p_3}$

	Transition rate for p_3
Into p_0	$\left(\frac{dq_3}{dt}\right)^- \frac{p_3}{p_3+p_5}$
Into p_5	$\left(\frac{dq_1}{dt}\right)^+ \frac{p_3}{p_0+p_2+p_3}$

	Transition rate for p_4
Into p_1	$\left(\frac{dq_2}{dt}\right)^- \frac{p_4}{p_2+p_4}$
Into p_2	$\left(\frac{dq_1}{dt}\right)^- \frac{p_4}{p_1+p_4p_5}$

	Transition rate for p_5
Into p_1	$\left(\frac{dq_3}{dt}\right)^- \frac{p_5}{p_3+p_5}$
Into p_3	$\left(\frac{dq_1}{dt}\right)^- \frac{p_5}{p_1+p_4+p_5}$

We can now write out the six additional equations for the fraction of cells in each state. For brevity, instead of writing

$$\frac{V}{M} r_1\left(f_1\left(c_1\right)-q_1\right),$$

we will simply write $\frac{dq_1}{dt}$. The equations are:

$$\begin{aligned}\frac{dp_0}{dt} =& -\Big(\frac{dq_1}{dt}\Big)^+\frac{p_0}{p_0+p_2+p_3} - \Big(\frac{dq_2}{dt}\Big)^+\frac{p_0}{p_0+p_1} - \Big(\frac{dq_3}{dt}\Big)^+\frac{p_0}{p_0+p_1}\\ &+ \Big(\frac{dq_1}{dt}\Big)^-\frac{p_1}{p_1+p_4+p_5} + \Big(\frac{dq_2}{dt}\Big)^-\frac{p_2}{p_2+p_4} + \Big(\frac{dq_3}{dt}\Big)^-\frac{p_3}{p_3+p_5},\end{aligned}$$

$$\begin{aligned}\frac{dp_1}{dt} =& \Big(\frac{dq_1}{dt}\Big)^+\frac{p_0}{p_0+p_2+p_3} - \Big(\frac{dq_1}{dt}\Big)^-\frac{p_1}{p_1+p_4+p_5} - \Big(\frac{dq_2}{dt}\Big)^+\frac{p_1}{p_0+p_1}\\ &- \Big(\frac{dq_3}{dt}\Big)^+\frac{p_0}{p_0+p_1} + \Big(\frac{dq_2}{dt}\Big)^-\frac{p_4}{p_2+p_4} + \Big(\frac{dq_3}{dt}\Big)^-\frac{p_5}{p_3+p_5},\end{aligned}$$

$$\begin{aligned}\frac{dp_2}{dt} =& \Big(\frac{dq_2}{dt}\Big)^+\frac{p_0}{p_0+p_1} - \Big(\frac{dq_2}{dt}\Big)^-\frac{p_2}{p_2+p_4} - \Big(\frac{dq_1}{dt}\Big)^+\frac{p_2}{p_0+p_2+p_3}\\ &+ \Big(\frac{dq_1}{dt}\Big)^-\frac{p_5}{p_1+p_4+p_5}\end{aligned}$$

$$
\begin{aligned}
\frac{dp_3}{dt} &= \big(\frac{dq_3}{dt}\big)^+ \frac{p_0}{p_0+p_1} - \big(\frac{dq_3}{dt}\big)^- \frac{p_3}{p_3+p_5} - \big(\frac{dq_1}{dt}\big)^+ \frac{p_3}{p_0+p_2+p_3} \\
&\quad + \big(\frac{dq_1}{dt}\big)^- \frac{p_5}{p_1+p_4+p_5}, \qquad (3.1)\\
\frac{dp_4}{dt} &= \big(\frac{dq_2}{dt}\big)^+ \frac{p_1}{p_0+p_1} + \big(\frac{dq_1}{dt}\big)^+ \frac{p_2}{p_0+p_2+p_3} - \big(\frac{dq_2}{dt}\big)^- \frac{p_4}{p_2+p_4} \\
&\quad - \big(\frac{dq_1}{dt}\big)^- \frac{p_4}{p_1+p_4p_5} \\
\frac{dp_5}{dt} &= \big(\frac{dq_3}{dt}\big)^+ \frac{p_1}{p_0+p_1} + \big(\frac{dq_1}{dt}\big)^+ \frac{p_3}{p_0+p_2+p_3} - \big(\frac{dq_3}{dt}\big)^- \frac{p_5}{p_3+p_5} \\
&\quad - \big(\frac{dq_1}{dt}\big)^- \frac{p_5}{p_1+p_4+p_5}.
\end{aligned}
$$

The IL-6 production only occurs in cells in class p_4 and p_5 with the production rate for class p_5 about 5 times as high as for class p_4 [3]. So IL-6 production is proportional to

$$p_4 + 5p_5.$$

The book keeping equations are purely for the short term. After the equilibrium values for q_1, q_2, and q_3 are reached, we expect the cell classes to equilibrate according to the expected probability distribution. That is, after a longer period of time, with the rates of desorption and resorption balancing out, we will expect

$$
\begin{aligned}
p_4 &= q_1q_2, \\
p_5 &= q_1q_3.
\end{aligned}
$$

4. Mathematical results for the model

We start by considering existence and uniqueness for (2.1). If we assume the f_j are Lipschitz, the Picard theorem [2] yields the following.

Theorem 4.1. *The system* (2.1) *has a unique local solution for each set of initial conditions. Furthermore, if the solutions stay bounded on each interval of existence, the system* (2.1) *has a unique global solution.*

There will be a difficulty for the Langmuir-Freundlich functions proposed above at $c_j = 0$ if any of the $\pi_j < 1$. However, we will show that when the initial conditions are strictly positive, then the solutions remain bounded and strictly positive, so the above theorem holds for every choice of positive initial conditions.

If we take the following masses per mole

Transcription factor	mass per mole
$AP-1$	m_1
$NF-\kappa B$	m_2
$AP-1-NF-\kappa B$	m_1+m_2

we obtain the following result.

Theorem 4.2. *For any solution to* (2.1) *the quantity*

$$m_1Vc_1 + m_2Vc_2 + (m_1+m_2)Vc_3 + m_1Mq_1 + m_2Mq_2 + (m_1+m_2)Mq_3$$

is constant in time

Proof. Multiply the first equation in (2.1) by m_1V, the second equation by m_2V, the third equation by $(m_1+m_2)V$, the fourth equation by m_1M, the fifth equation by m_2M, and the sixth by $(m_1+m_2)M$ and then add all of the equations together we obtain

$$\frac{d}{dt}\left(m_1Vc_1+m_2Vc_2+(m_1+m_2)Vc_3+m_1Mq_1+m_2Mq_2+(m_1+m_2)Mq_3\right)=0,$$

and we are done. We will call that constant value H_1. □

We observe that if all of the components of the solution are nonnegative, than the above result implies each component will be bounded by some positive constant, G. With this observation, we now obtain positivity of solution for positive initial conditions.

Theorem 4.3. *Assume that there is are positive constant K and $p \in (0,1]$ so that for positive c_j in a neighborhood of 0, $f_j \le Kc_j^p$. For any solution to (2.1), if all of the initial conditions are positive, then the solutions will remain positive. Given the previous result, any such solution will be confined to a bounded region in the positive cone of $\mathbb{R}^6$.*

Proof. We start with the observation that for each j,

$$\frac{dq_j}{dt} \ge -\frac{V}{M}r_jq_j,$$

Thus

$$q_j(t) \ge \exp\big(-\frac{V}{M}r_jt\big)q_j(0) > 0.$$

Because any solution will be continuous on its interval of existence, there will be a interval on which all components are nonnegative. Let $[0,T]$ be the maximum such interval. Let $t \in [0,T]$; then

$$\begin{aligned}\frac{dc_1}{dt} &= r_1\left(q_1 - f_1\left(c_1\right)\right) - k_1c_1c_2 + k_2c_3\\ &\ge r_1\exp\big(-\frac{V}{M}rt\big)q_1(0) - r_1Kc_1^p - k_1Gc_1.\end{aligned}$$

Thus, if $r_1\exp\left(-\frac{V}{M}rt\right)q_1(0) > r_1Kc_1^p + k_1Gc_1$,

$$\frac{dc_1}{dt} > 0$$

and c_1 has a positive lower bound on the entire interval. A similar argument provides positive lower bounds for c_2 and c_3 on the whole interval $[0,T]$. Therefore, the interval is actually infinite and the solutions components are strictly positive for all positive t. □

We will now obtain two more conservation relationships that will allow us to state what the unique equilibrium will be for a given set of positive initial values.

Theorem 4.4. *For any solution to (2.1) the quantities*

$$Vc_1 + Mq_1 + Vc_3 + Mq_3$$

and

$$Vc_2 + Mq_2 + Vc_3 + Mq_3$$

are constant. We will call these constants H_2 and H_3 respectively

Proof. To obtain the first, multiply the first equation in (2.1) by V the third equation by V, the fourth equation by M,and the sixth by M and then add all of the equations together we obtain

$$\frac{d}{dt}\left(Vc_1 + Vc_3 + Mq_1 + Mq_3\right) = 0.$$

The second sum is analyzed the same way. □

We now see that we have an equilibrium if the following equations are satisfied

$$\begin{gathered} m_1Vc_1 + m_2Vc_2 + (m_1+m_2)Vc_3 \\ + m_1Mq_1 + m_2Mq_2 + (m_1+m_2)Mq_3 = H_1, \\ Vc_1 + Mq_1 + Vc_3 + Mq_3 = H_2, \\ Vc_2 + Mq_2 + Vc_3 + Mq_3 = H_3, \\ q_1 = f\left(c_1\right), \\ q_2 = f_2\left(c_2, c_3\right), \\ q_3 = f_3\left(c_2, c_3\right), \\ c_3 = \frac{k_1}{k_2}c_1c_2, \end{gathered}$$

The monotonicity properties of the f_j should guarantee a unique positive equilibrium point.

Turning to the book keeping equations, (3.1) the observation that the forcing functions are Lipschitz in the p_j and continuous in t will allow the application of the Picard theorem again to guarantee unique local solutions. Simply adding the equations up will yield

$$\frac{d}{dt}\left(p_0 + p_1 + p_2 + p_3 + p_4 + p_5\right) = 0;$$

i.e., the total number of cells is conserved by the model. Similar arguments as the ones used for (2.1) will give us that the cell classes stay nonnegative.

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