Developing a model of Cro regulation of early CI production in bacteriophage lambda infections.

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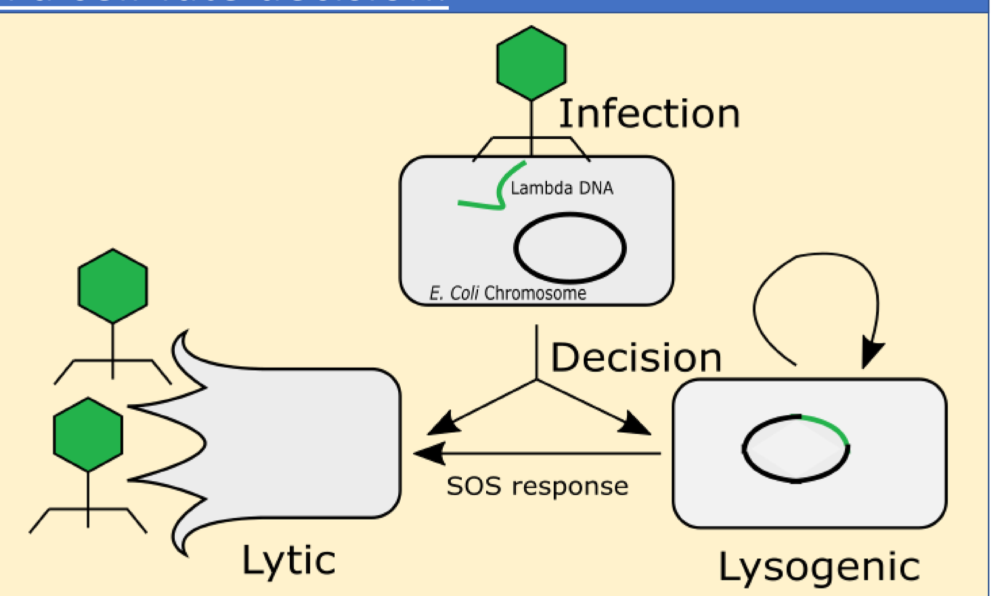
Lambda infection results in a cell-fate decision.

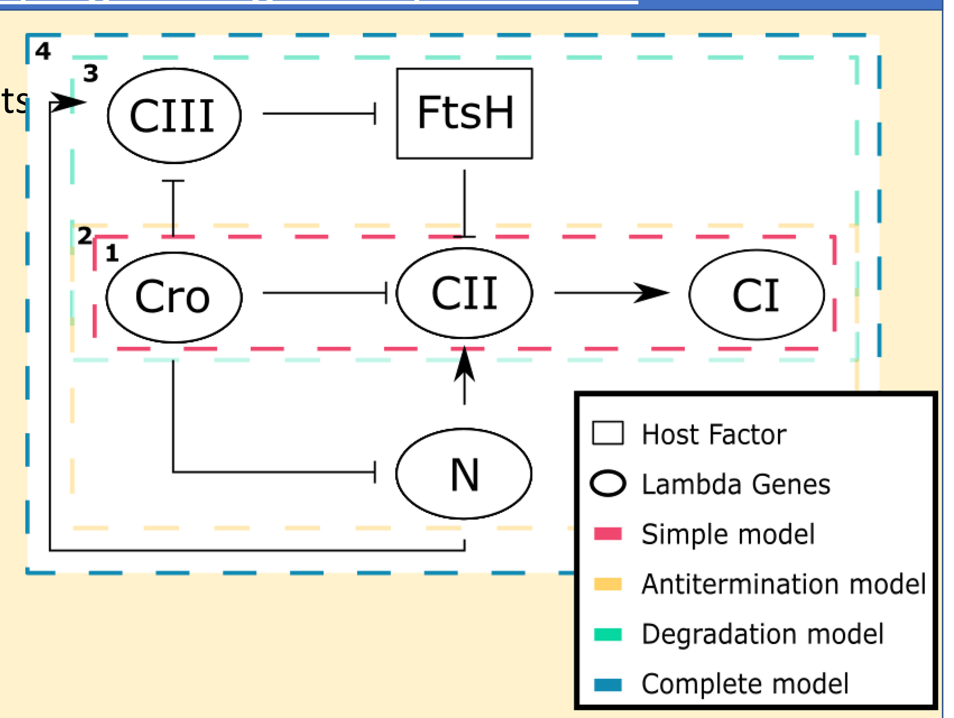
- Lambda is a virus which infects E. coli cells, resulting in a cell-fate decision – a binary choice between two possible phenotypic outcomes.[1]
- The decision outcome is biased by numerous factors (such as the number of infecting viruses) and is governed by a complex regulatory network.



Infection decision cycle.
The virus injects genetic material to the host. A decision occurs between lytic and lysogenic pathways. In the stable lysogenic state genetic material is stored and maintained through generations. In environment stress and SOS response can lead to lysis. In the lytic pathway the virus reproduces and kills the host.

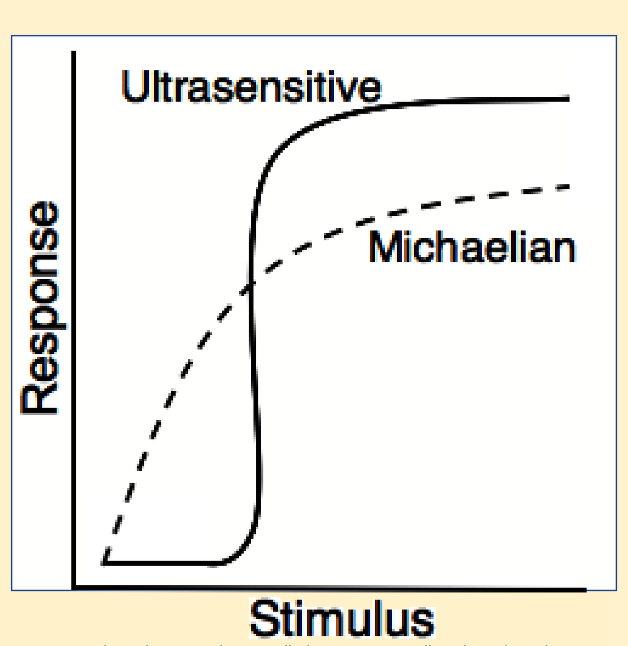
The decision is determined by a gene regulatory network.

- CI establishes and maintains the lysogenic state, while Cro prevents expression of CI and the establishment of the lysogenic state. [2]
- Cro binds relatively weakly and noncooperatively, while CI binds strongly and forms oligomeric structures.
- It's not well understood how Cro can exactly prevent the establishment of the lysogenic pathway.
- Cro influence in early CI expression may lie in the fact that it regulates this expression in multiple ways through a gene regulatory network.



Schematic of gene regulatory network in lambda.
Simple model includes a direct Cro, CI interactions. Antitermination model includes the simple model and the N anti-terminator. Degradation model includes the simple model and the interactions between CIII and FtsH protease.

R as a measurement of strength of Cro early influence on CI.



Wikipedia contributors. "Ultrasensitivity." *Wikipedia, The Free Encyclopedia*. Wikipedia, The Free Encyclopedia, 11 Jun. 2019. Web. 24 Jul. 2019.

Ultrasensitivity.
An Ultrasensitive response is more sensitive than classical Michaelis – Menten kinetics.

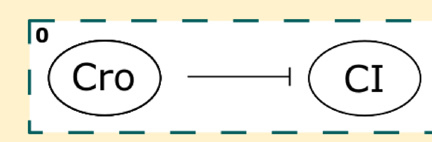
- Ultrasensitivity can be described as a biological switch.
- We use |R|max of each model to measure the strength of the response of CI at different concentrations of Cro.

$$R = \frac{dCI}{dCro}\frac{Cro}{CI}$$

- Mathematically, a system is said to be ultrasensitive if |R|>1.

Analyzing the strength of response of each model.

0^{th} model

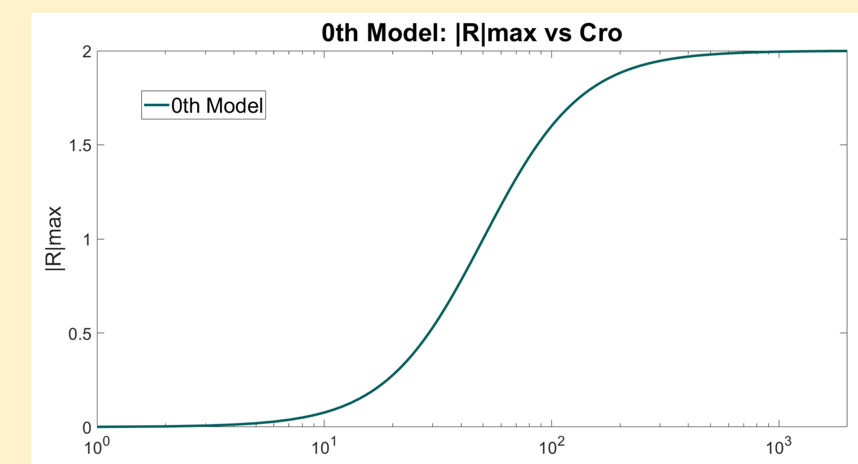


Change in CI concentration over time

CI Degradation rate

$$\frac{dCI}{dt} = r_{CI}\left(\frac{K_{CI}^{nCI}}{K_{CI}^{nCI} + Cro^{nCI}}\right) - k_{CI}CI$$

Production rate of CI



In a scenario where Cro directly represses CI expression, it can be observed how the strength of the response varies in a given Cro concentration.

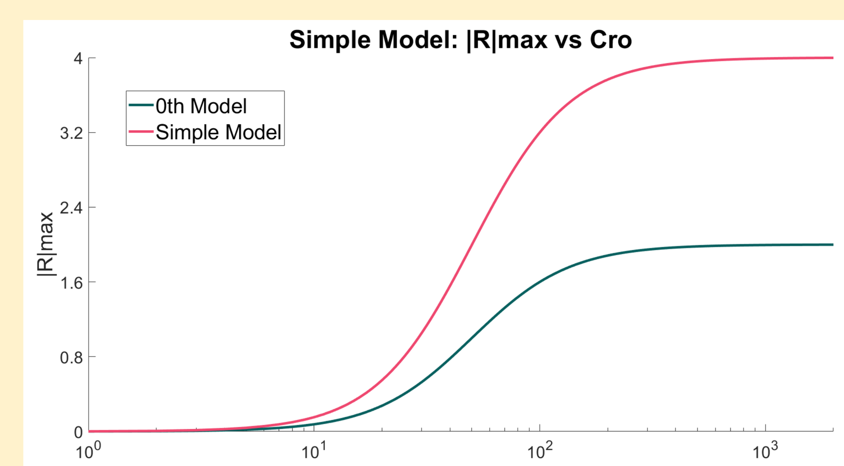
Simple model

$$\frac{dCII}{dt} = r_{CII}\left(\frac{K_{CII}^{nCII}}{K_{CII}^{nCII} + Cro^{nCII}}\right) - k_{CII}CII$$

Production rate of CI

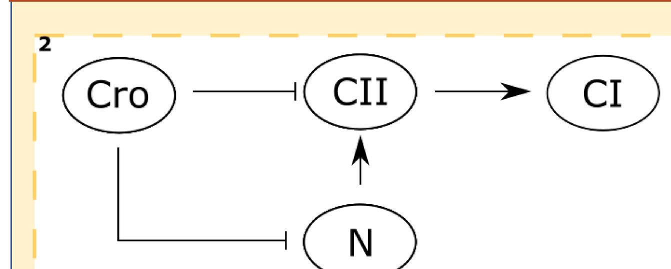
$$\frac{dCI}{dt} = r_{CI}\left(\frac{CII^{nCI}}{CII^{nCI} + K_{CI}^{nCI}}\right) - k_{CI}CI$$

Activation of CI production by CII



CII is responsible of activating CI transcription, and Cro represses CII production, therefore, when CII module is introduced the simple model has a stronger response.

Antitermination model

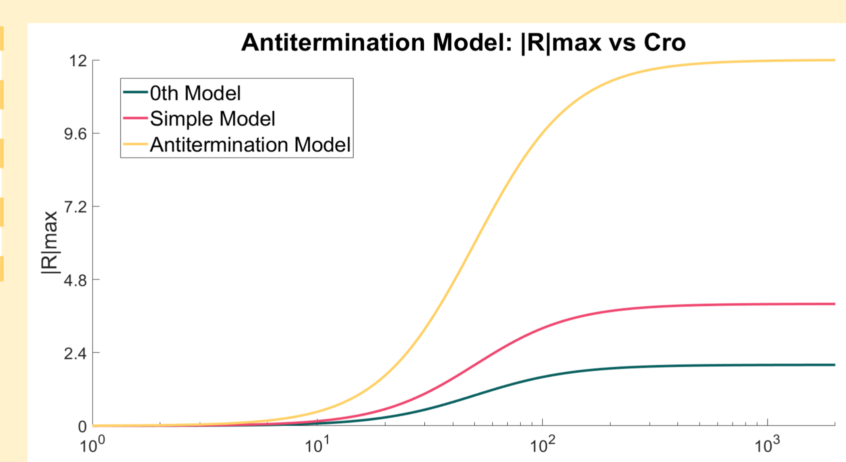


$$\frac{dN}{dt} = r_N\left(\frac{K_N^{nN}}{K_N^{nN} + Cro^{nN}}\right) - k_N N$$

Production rate of N

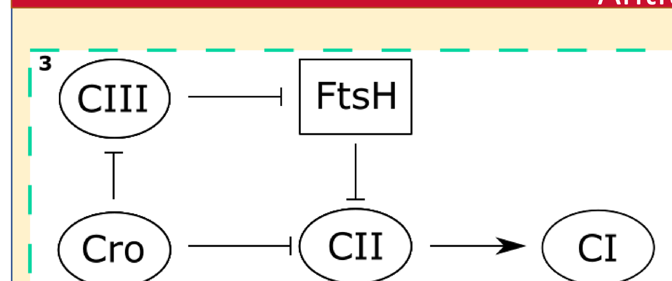
Activation of CII production by N

$$\frac{dCII}{dt} = r_{CII}\left(\frac{K_{CII}^{nCII}}{K_{CII}^{nCII} + Cro^{nCII}}\right)\left(\frac{N^{nN}}{N^{nN} + K_N^{nN}}\right) - k_{CII}CII$$



The antiterminator N has to be present so transcription can proceed, making the production of CII available. Since Cro represses both, the response is even greater.

Antitermination model



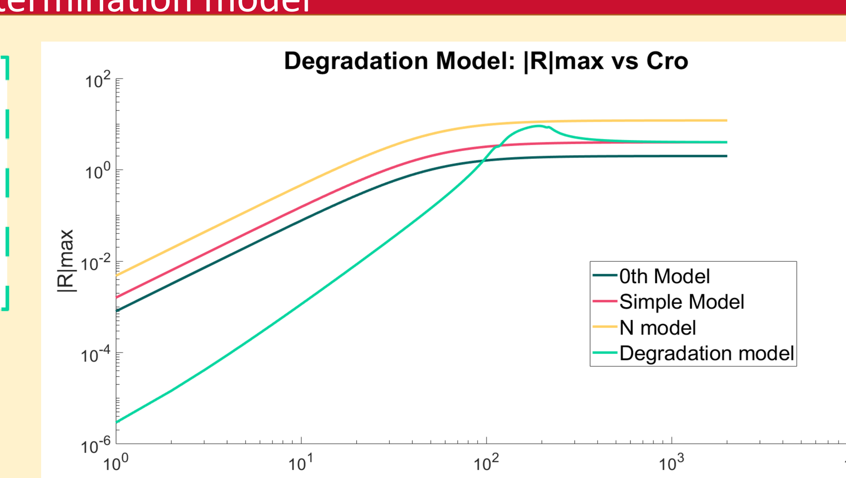
$$CII + FtsH \leftrightharpoons C_1 \rightarrow \alpha_1 + FtsH$$
$$CIII + FtsH \leftrightharpoons C_2 \rightarrow \alpha_2 + FtsH$$

Production rate of CII

Modified degradation of CII rate from enzyme kinetics analysis

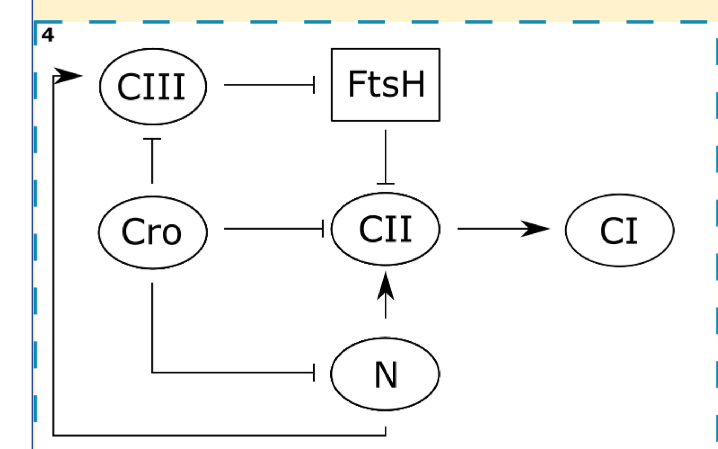
$$\frac{dCII}{dt} = r_{CII}\left(\frac{K_{CII}^{nCII}}{K_{CII}^{nCII} + Cro^{nCII}}\right) - \frac{\frac{k_{CII}}{K_{CII,FtsH}}CII}{1 + \frac{CII}{K_{CII,FtsH}} + \frac{CIII}{K_{CIII,FtsH}}}$$

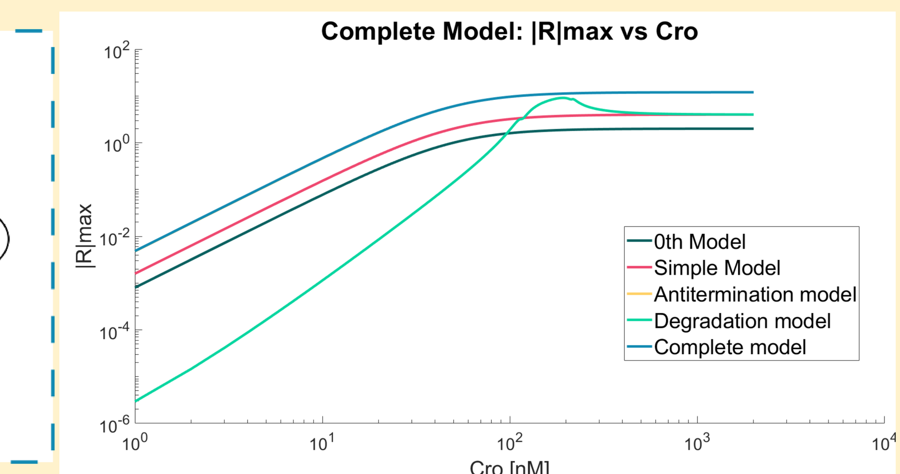
$$\frac{dCIII}{dt} = r_{CIII}\left(\frac{K_{CIII}^{nCIII}}{K_{CIII}^{nCIII} + Cro^{nCIII}}\right) - \frac{\frac{k_{CIII}}{K_{CIII,FtsH}}CIII}{1 + \frac{CII}{K_{CII,FtsH}} + \frac{CIII}{K_{CIII,FtsH}}}$$



The current understanding tells us that FtsH is a protease which degrades CII and CIII. We use Michaelis-Menten enzyme kinetics to get a new degradation rate.

Antitermination model





In the last model we combine all models taking into account all the possible ways Cro can influence CI.

$$\frac{dCII}{dt} = r_{CII}\left(\frac{K_{CII}^{nCII}}{K_{CII}^{nCII} + Cro^{nCII}}\right)\left(\frac{N^{nN}}{N^{nN} + K_N^{nN}}\right) - \frac{\frac{k_{CII}}{K_{CII,FtsH}}CII}{1 + \frac{CII}{K_{CII,FtsH}} + \frac{CIII}{K_{CIII,FtsH}}}$$

Activation of CIII production by N

$$\frac{dCIII}{dt} = r_{CIII}\left(\frac{K_{CIII}^{nCIII}}{K_{CIII}^{nCIII} + Cro^{nCIII}}\right)\left(\frac{N^{nN}}{N^{nN} + K_N^{nN}}\right) - \frac{\frac{k_{CIII}}{K_{CIII,FtsH}}CIII}{1 + \frac{CII}{K_{CII,FtsH}} + \frac{CIII}{K_{CIII,FtsH}}}$$

Production rate of CIII

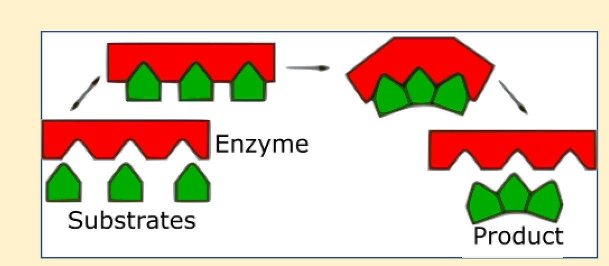
Modified degradation of CIII rate from enzyme kinetics analysis

Results/Discussion.

- Simple, Antitermination and Complete models show ultrasensitive response for a large variety of parameters.
- Using Simple Michaelis – Menten kinetics mathematical analysis is not sufficient to achieve ultra sensitivity. A model involving cooperativity might be needed.

Future work.

The degradation model of the network is likely to exhibit a strong ultrasensitive response. More complex analysis is needed to taking into account cooperativity. Early work done in this have shown a promising strong response, but optimization is still needed.
The development of these models was considered on Steady State. Now, we are looking to incorporate the time dynamics to our models, creating a mechanistic model which we can compare to experimental data.



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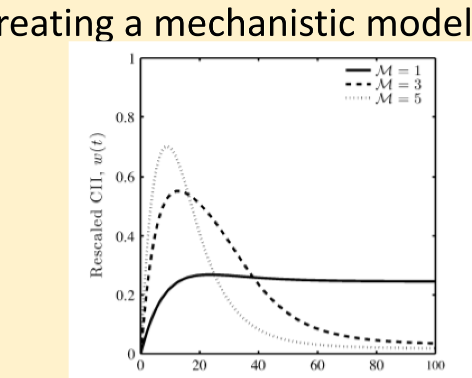

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[2] Oppenheim AB, Kobiler O, Stavans J, Court DL, Adhya SL. 2005. Switches in bacteriophage lambda development. Annu. Rev. Genet. 39:409-29

Acknowledgments.

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