Large-scale stability analysis of the p53-MDM2-ARF axis in neuroblastoma

Rory Deignan[1,2], Melody Parker[2], Dawn C. Walker[2,3], Kenneth Y. Wertheim[2,3]

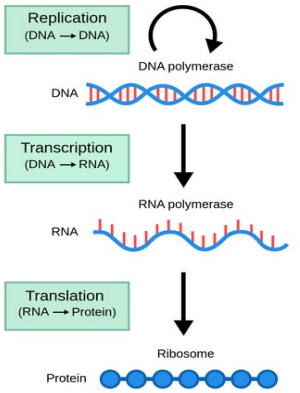
1. Department of Physics and Astronomy, University of Sheffield, 2. Insigneo Institute for In-Silico Medicine,
3. Department of Computer Science, University of Sheffield

Abstract

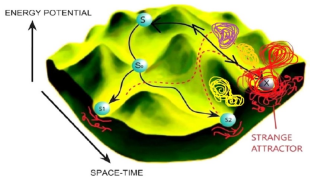
The MDM2-p53-ARF genetic network is a system of genes that are known to be important in repairing or terminating a stressed cell [4]. In particular, the p53 gene is responsible for apoptosis, which can cause tumor regression in various cancers. Using an ordinary differential equation (ODE) model, we investigated the long-term behaviour of a the axis, with a focus on how it is affected by MYCN amplification. Low-risk patients can be effectively treated for neuroblastoma, and high MYCN protein is a positive indicator; however, high-risk patients have poor five-year survival rates, and MYCN overamplification in DNA is a high-risk classifier). We searched the parameter space of the genetic network to study which other factors could induce better outcomes, and where the bifurcation in cell fate occurs. The model was too non-linear to perform a global stability analysis, and likewise the bifurcation could not be mapped to a single control parameter. However, we found there were qualitatively distinguishable varieties of parameter sets; there was only ever one attracting fixed point (energy minima) in a parametric space, or none at all. This agrees with other fundamental theories in mathematical biology [3][5], hence supporting the validity of the model.

Introduction

The central difficulty in understanding the behaviour of the p53-MDM2-ARF axis is its non-linearity. There is a well documented feedback loop between MDM2 and p53 [3], which is present even on the mRNA level. p53 is also self-promoting, and is further promoted by MYCN. This initially implies that high MYCN concentration in cancerous cells is beneficial, however when MYCN is overamplified as a result of genetic factors, patient outcomes are very poor (~9% five-year survival). The ODE model by Melody Parker [2] converts principles from the Central Dogma of Molecular Biology to mathematical formalism (graphic upper left). To handle the difficulty of taking real measurements of gene concentrations, the model is scaled, in order to describe the qualitative features of the axis. Two 'layers', for mRNA and DNA, dictate the protein levels over time of the four genetic species under study (examples above). We interpret the energy minima (or attracting fixed-point solutions) of the model as phenotypes of the cell, based on the theory of Waddington's Epigenetic landscape (graphic left [3]). Changing parameters in the model and studying how fixed-point solutions change as a result can tell us which parameters affect the phenotype of the cell i.e. its long-term fate. Mathematically this is known as bifurcation. Of particular interest is the Hopf bifurcation, when a fixed point gives birth to a limit cycle in its neighbourhood. It would be very useful to know where the limit cycle associated with p53 up-regulation appears, for example. Other long term behaviours, like quasi-periodicity and chaotic oscillation, can represent various cell fates [5].



[Fig 1, above] A visualization of the Central Dogma of Molecular Biology. The two arrows can be thought of as the two rates of change for each gene in the model



[Fig 2, above] A visualization of Waddington's Epigenetic landscape, with potential energy minima marked as fixed points. From [3]

$$\frac{d}{dt}[MYCN_m] = g_{MYCN}\{\phi_{MYCN}\ \alpha_{MYCN} + (1-\phi_{MYCN})\ \beta_{MYCN}\} - \{\varphi_{MYCN}\ d_{MYCN_m} + (1-\varphi_{MYCN})\, d_{MYCN_m-G}\}[MYCN_m]$$

$$\frac{d}{dt}[MYCN] = \lambda_{MYCN}[MYCN_m] - \{\theta_{MYCN}\ d_{MYCN} + (1-\theta_{MYCN})\ d_{MYCN-A}\}[MYCN]$$

$$\frac{d}{dt}[P53_m] = g_{P53}\{\phi_{P53}\ \alpha_{P53} + (1-\phi_{P53})\beta_{P53}\} - d_{P53_m}[P53_m]$$

$$\frac{d}{dt}[P53] = \{\sigma_{P53}\lambda_{P53} + (1-\sigma_{P53})\ \mu_{P53}\}[P53_m] - \theta_{P53}\left\{d_{P53} + \left(\frac{(1-s_4S)\ d_{P53-C}}{K_C} + \frac{(1-s_5S)\ d_{P53-D}}{K_D}\right)[MDM2] + \frac{(1-s_5S)\ d_{P53-F}}{K_F}[ARF][MDM2]\right\}[P53]$$

[Fig 3, left] The equations for MDM2 and p53 mRNA and DNA production rates from Melody Parker's model. Each term underlined in cyan is a fraction of protein in a certain state, itself a dynamic variable dependent on the other gene concentrations. From [2]

Methodology

Latin hypercube (LHC) sampling was used sample the 30-dimensional parameter space of the model as evenly as possible. For each combination of parameters, the fixed points were found using the Newton-Raphson root finding method. This method is dependent on the initial guess we use in the formula, so we needed to develop a heuristic to decide when we should keep sampling the space, and when to stop. The search was designed to exhaust itself after failing to find new points many times in a row, or after a maximum number of total guesses had been exceeded. The code for the study was written in Python, and mainly used the popular SciPy and Numpy packages. Classification was performed by running automated dynamic simulations on every fixed point, as the typical Jacobian stability analysis failed to produce verifiable results. This is due to the highly non-linear nature of the network, which renders it insoluble via many analytical methods that rely on linear approximations.

We also built and successfully tested the code framework to measure the dimensionality of various periodic systems and intend to use these methods, along with Discrete Fourier Transform (DFT)/power spectra analysis to classify the different dynamics that are possible within each parameter set.

References

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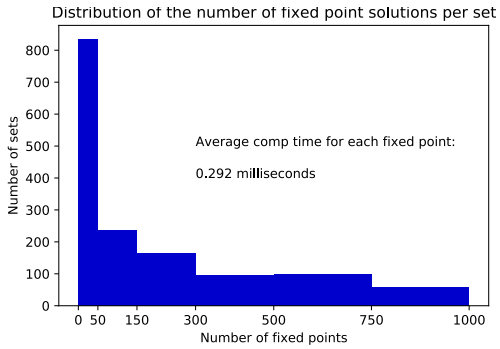
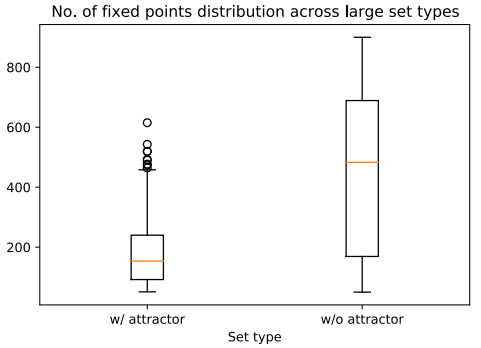
Results

The fixed-point search did not exhaust as many parameter sets as we planned, due to the intensity of computation and time constraints. However, the distribution of the number of fixed points found in each set, and whether the fixed-points were attractors (energy minima) or repulsors (maxima) was found to be weakly correlated. More promisingly, we noted the presence of either one attractor or none in every set. We were also able to verify that the code for our dimensional analysis worked, by testing it on example models. We successfully measured the correlation dimension of two different limit cycles, normal and quasi-periodic. We did the same for a fractal object, the Cantor Set, to be sure that our analysis could be applied to strange attractors as well as integer dimension behaviours.



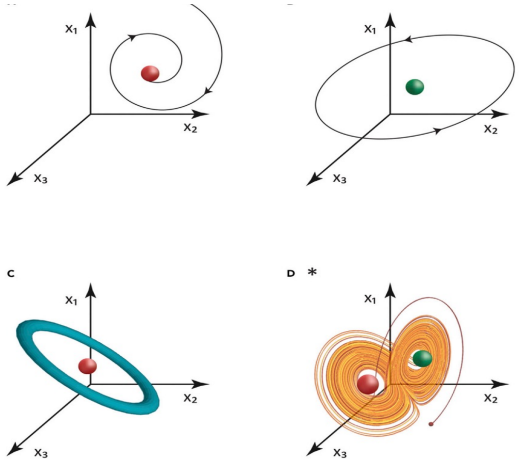
[Fig 4] Histogram to show the distribution of the number of fixed points found in each parametric combination. Overall 1500 sets were tested.



[Fig 5] Box plot of the distribution of fixed point set sizes, for sets with more than 50 fixed points, distinguished by the presence or absence of an attractor in the set.

Discussion

The results seem to align with Waddington's theory, as only attractors allow protein levels to settle long enough to produce a stable phenotype. Furthermore, if a set of fixed points only contains repulsors, it is more likely to sustain periodic oscillations. These could either be regular/quasi-periodic oscillatory e.g. limit cycles associated with the p53-MDM2 apoptosis cycle [4], or chaotic behaviours, which have been previously theorized to represent malignant cancer stem-cell qualities [5]. Hence, somewhere in the parameter space, the system can bifurcate from having one stable phenotype to exhibiting a range of behaviours in response to cell stress. The next phase of development for the project is to study the long-term behaviour of the genetic network e.g. limit cycles, quasi-periodicity, and chaos (graphic left [3]). This will be guided by expanded data from the fixed-point search and classification process for many more parametric combinations. Furthermore, we could extend the bifurcation analysis of the parameter sets with Principle Component Analysis. This collapses different dimensions in the model into one, to identify highly non-linear bifurcations where several factors are involved.



[Fig 5] Visualizations of different long-term behaviours in a parameter space. A) Attracting fixed point, B) Limit cycle, C) Quasi-periodic limit cycle, D) Strange attractor. The shift from A to B would be known as a Hopf bifurcation From [3]