COMPUTATIONAL ANALYSIS OF THE RB-E2F PATHWAY:

CLASSIFICATION OF PARAMETERS

By

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Abstract

The restriction point, or R-point, of the cell cycle behaves as a switch-like system which controls whether a cell progresses from the G1 phase to the S phase. The mechanism which controls the restriction point has been determined to be the Rb-E2F pathway. The Rb-E2F pathway is bistable; this allows for the system to exist in two states, ON or OFF, and explains the switch-like behavior observed in the R-point. The stimulus of this system is serum growth signal which exist at a concentration either low enough to keep the cells in a non-growing state called cellular quiescence or at a high level that activates the pathway into the ON state and commits the cell into proliferation. Yao et al. constructed a mathematical model to analyze the dynamics of the Rb-E2F pathway. The research of this paper involves the classification of the parameters used in this mathematical model to better understand similarities and differences of the components within the network's topology. Once classified, further analysis was performed with parameters that resulted in the decrease of bistable width. Only two phenotypes were observed from this analysis which suggests some potential evolutionary advantages of the bistable system.

Introduction

The importance of understanding the mechanisms behind the cellular commitment to proliferate is to gain insight about how proliferative diseases such as cancer arise. Being able to assemble a model that predicts which genes create disease-like phenotypes is an incredible tool for potential therapeutics. In normal cells, the restriction point acts as a checkpoint to regulate whether the cell will go through proliferation or remain in a quiescent state. Research has demonstrated that in virtually all cancers, the regulation of the restriction point has been disrupted (2). Cancerous cells become able to bypass the state of quiescence and maintain a constant driving force for over-proliferation (4).

The relationship between the restriction point and the Rb-E2F gene network becomes critical in understanding why this phenomenon occurs. The Rb-E2F pathway controls how cells in the quiescent state transition to the proliferative state by activation of the transcriptional factor E2F. E2F positively regulates genes that are linked to cell cycle progression. Yao et al. has confirmed that the activity of this pathway is based upon a bistable system. Bistability is defined in dynamic systems as having two stable steady-states. The Rb-E2F pathway has two states: E2F-ON and E2F-OFF. When there is enough stimulus from serum growth signals, the cell becomes E2F-ON and is able to pass the restriction point. The amount of stimulus necessary to activate the ON state is called the OFF-ON threshold. The cell remains in the E2F-ON state until the stimulus is reduced to a certain extent. This extent is called the ON-OFF threshold. This is conceptually illustrated in Figure 1 from Yao et al in 2011 (6). The observed behavior is indicative of an all-or-nothing response. Alternatively, when the pathway is in the E2F-OFF state, the cell remains in quiescence until there is enough stimulus to change states.

Yao et al. has constructed a simplified mathematical model of the Rb-E2F pathway and the output of this model is E2F. A schematic model of this pathway is reproduced from Yao et al. 2008 in Figure 2 (5). The ON/OFF mechanism of E2F ultimately decides whether the genes that are associated with the processes of DNA replication and cell cycle progression become activated. During quiescence, cells express the OFF form of E2F. This OFF state is regulated by the expression of active Rb, or retinoblastoma. Retinoblastoma is a protein which when active, interacts and binds to E2F (3). This creates an Rb-E2F complex that represses the normal function of E2F. However with sufficient serum growth signals, the function of Rb is inhibited through phosphorylation of Rb by cyclin-Cdk complexes. In this pathway, cyclin D-Cdk4,6 (CycD-Cdk4,6 for short) and cyclin E-Cdk2 (CycE-Cdk2) are the complexes that phosphorylate and repress the active Rb function (3). Upstream of the formation of these cyclin-Cdk complexes is Myc, which is a proto-oncogene that is activated with the induction of serum growth signals (1). Activated Myc promotes both cyclin D production as well as E2F transcription which results in cyclin E production. Therefore, the presence of sufficient amounts of stimulus results in this cascade of signaling that represses Rb. When Rb is repressed, the Rb-E2F complex is no longer able to be maintained and E2F becomes active again (3).

The Yao model takes into account all of these events involved in the Rb-E2F pathway in a simplified manner through seven ordinary differential equations (ODEs) and twenty-five base parameters. The 25 base parameters are portrayed schematically in Figure 3 and the variables are defined in Table 1 (5). The differential equations that are fundamental to the model are shown in Table 2 (5). Previous analysis has been performed with this model by Everetts, a member of the Yao lab, which demonstrated simulations of how certain system characteristics were affected by increasing or decreasing single parameters. Everetts analyzed the sensitivity of the response from

these parameter perturbations and created a series of spider graphs for the simulations as seen in Figure 4 (2). The purpose of this paper is to analyze and classify four of the series of simulations into tangible and coherent categories; the included series are the OFF-ON threshold, ON-OFF threshold, bistable width, and bistable midpoint. Bistable width is a measurement of the range of serum concentrations that is able to maintain E2F activation. Bistable midpoint defines the position of the range. Together, bistable width and midpoint represent the bistable region in terms of serum concentration. The initial classification of these observations serves as a foundation to speculate similarities and differences amongst the parameters in the network. Further analysis is performed for bistable width to observe which parameter changes result in decreases to bistable width. The significance of this analysis is to further elucidate which perturbations lead to a loss of bistability, or monostability. These model predictions can then be used to form testable hypotheses for "proof of principle" experiments to further validate the Yao model.

Methods

Parameter sensitivity simulations through COPASI

The computer program called COPASI, or COmplex PAthway SImulator, was utilized as a tool to run various simulations of the pathway at different levels of serum growth signals and to observe how "mutations" or parameter perturbations would affect the output. A task called Parameter Scan was run with the Yao model to get a wild-type simulation of values, or base values, at serum inputs between 0 and 20% in intervals of 0.2%. The concentrations of the various species and the rates of the reactions were determined by the values reported by Yao et al in 2008 with some recently edited values. Each of the 25 parameters were then perturbed individually by a tenfold factor both increase and decrease. Parameter Scans were run again with these perturbations and the change compared to the wild-type simulation were recorded. This process was done with the four series of simulations: the OFF-ON threshold, ON-OFF threshold, bistable width, and bistable midpoint. The factor change from the parameter perturbations would signify the sensitivity of that parameter; a drastic factor change would indicate a highly sensitive parameter.

Graphical representation and categorization of single parameter perturbations

The obtained values from the COPASI simulations were plotted using Microsoft Excel for simple data management. The axes were set to logarithmic scale to better visualize the behavior of the changes occurring at the tenfold decrease factor change. This was initially done in the form of spider graphs for each series of simulation: OFF-ON threshold, ON-OFF threshold, bistable width, bistable midpoint, that included all 25 parameters by Everetts in 2015. This paper converted the spider graphs from Everetts 2015 into spider graphs that included the

data from the OFF-ON threshold, ON-OFF threshold, bistable width, and bistable midpoint for individual parameters. This essentially characterized each of the parameters which led to sorting them by similar behavior. Figure 5 depicts four of the categories and the graphs that correlate with their behavior.

Categorization based on four characteristics of the Rb-E2F model

The categorization of the graphs was done by assigning variables to the four characteristics as follows: X = OFF-ON threshold, Y = ON-OFF threshold, W = Bistable Width, M = Bistable Midpoint. These variables also had qualifiers to represent whether the behavior was occurring during a decrease of the parameter (left area of the graph) or an increase. The decrease is marked by L and the increase is marked by R. These variables were assigned a value of -1, 0, or 1. -1 represents a decrease in factor change, 0 signifies no change, and 1 represents an increase. Parameters that had the exact same values for all four characteristics were categorized into a single group. It is possible for certain combinations to not occur and it is also possible for some categories to only have one unique parameter in them.

Construction of schematic model for observations of bistable width decrease

From the original schematic presented by Yao et al in 2008 and then the parameter-included version by Everetts in 2015, a similar schematic was created to identify the behaviors of the Rb-E2F model when perturbations caused bistable width decrease. The green arrows represent an increase of parameter or that the behavior can be observed in the right area from the graphical data. The red arrows display the opposite, a decrease of parameter. The thickness of the arrows were based upon how drastic the factor change was, the thicker arrows indicating a larger

change. In the situation presented in this paper, the thicker arrows indicated a stronger decrease in bistable width.

Results and Discussion

The result from the classification process was the establishment of nine categories for the twenty-five parameters. The established categories were based off a system which utilized each of the four characteristics and then added a qualifier to whether the parameter was increased or decreased. Each of these specific characteristics were defined by three values. Mathematically, there are much more than 9 possible categories that could have been expressed; a question to be raised is why these nine. Do they confer some evolutionary advantage? What events led to cells preferring this kind of machinery? However, the focus of this paper is to try to gain insight about how the machinery for the Rb-E2F network behaves in a more narrow scope.

The classification of the parameters led to observing which parameters produced a decrease in bistable width. In Table 3, this would be indicated by the value of -1 in WL and/or WR. The behaviors of the OFF-ON threshold, ON-OFF threshold, and the bistable midpoint were all analyzed for these parameters. It was observed that there were only two phenotypes from parameters that decreased bistable width: one phenotype shifted the midpoint to the left, the other shifted the midpoint to the right. There were no cases of the midpoint staying near the base value and the bistable width decreasing. One observation from these two phenotypes was that both of the midpoint shifts only involved one threshold shifting strongly towards the other while the other remains in the relatively same location. Further analysis was performed with the parameters that were separated into these two phenotypes. It was interesting to discover that the parameters that were involved with shifting the midpoint to the left were all affecting the upstream components of the pathway near CycD-Cdk4,6 and Rb. This phenotype also had parameters that directly affected E2F activity. This observation was presented in Figure 6 in a schematic model including the parameters. The phenotype that shifted the midpoint to the right

also had the same kind of result: all of the parameters that were involved with this phenotype were located downstream of the Rb-E2F pathway in what is called the biomodule. Figure 7 illustrates this result.

The implication of the results from this analysis is that within the Rb-E2F network, the upstream components are mainly regulating the OFF-ON threshold while the downstream components are regulating the ON-OFF threshold. There may exist some other regulatory mechanisms that keeps the other threshold at near its base value as well. In reference to Figure 1, this would mean that the parameters involved in shifting the midpoint to the left would make the state of quiescence, or the E2F-OFF state shallower. The overall effect of this change is observed frequently in cancer. If the state of quiescence is easier to move out from, then cell proliferation will occur at a higher rate than normal. This was an observation made in previous experimental research too as cancer cells have demonstrated that they are able to bypass quiescence. Alternatively, the parameters involved in shifting the midpoint to the right would do the opposite and make the state of E2F-ON shallower. This implies that the maintenance of keeping the E2F-ON state would become more difficult. Thus, the parameters of the Rb-E2F model that decrease bistable width may contribute some mechanical/dynamic insight to disease-like phenotypes that occur due to a loss of bistability.

The future directions with research of the Rb-E2F pathway would include comprehensive analysis of the half-activation time of the parameters and the amplitude of the bistable region. Being able to simulate the entire bistable region may lead to new hypotheses how the restriction point operates. Also, any experiments that could test the predictions from the COPASI simulations would provide a “proof of principle” which ultimately validates and supports the Yao model.

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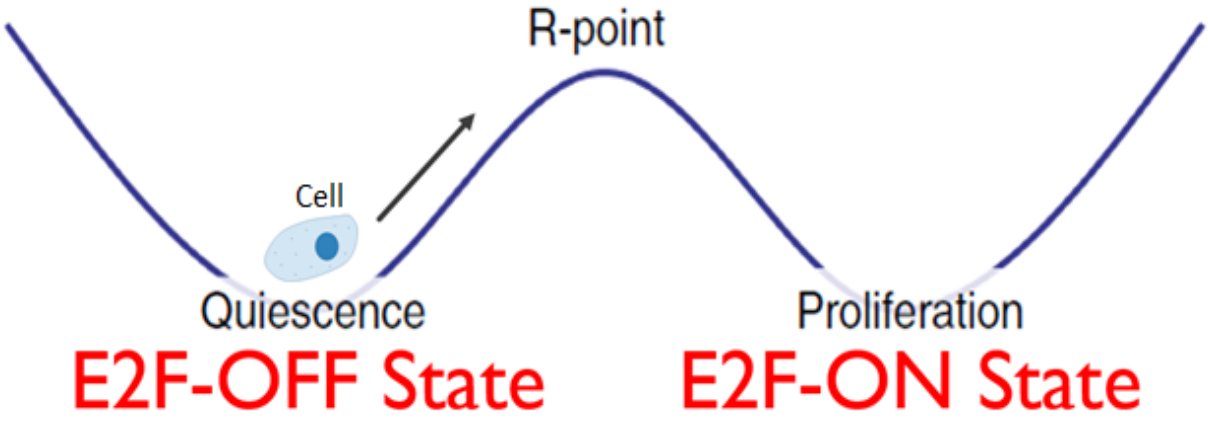

Figure 1. A schematic representation of the bistable system involved with the restriction point during G1 phase. There are two states, E2F-OFF and E2F-ON. The maxima of the curve is depicted as the R-point and portrays a threshold for activation into proliferation and de-activation into quiescence. Reproduced from Yao et al. 2011 (6)

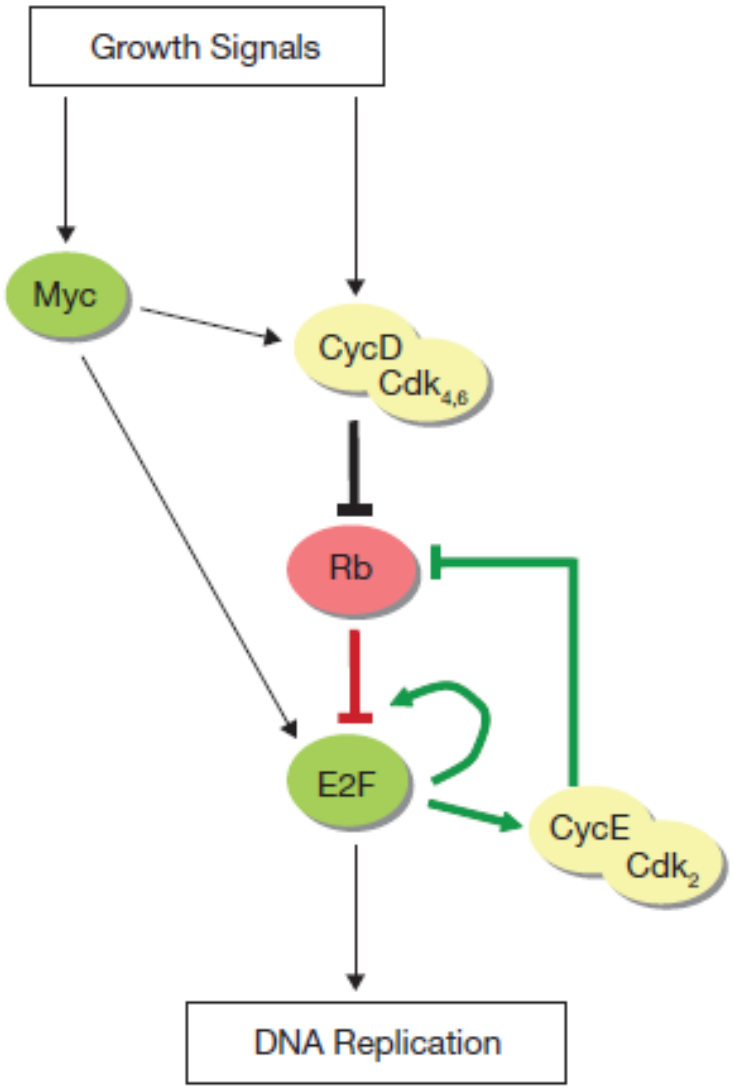

Figure 2. The simplified schematic model of the Rb-E2F pathway. The arrows demonstrate activation and the barred arrows depict repression. Reproduced from Yao et al. 2008 (5).

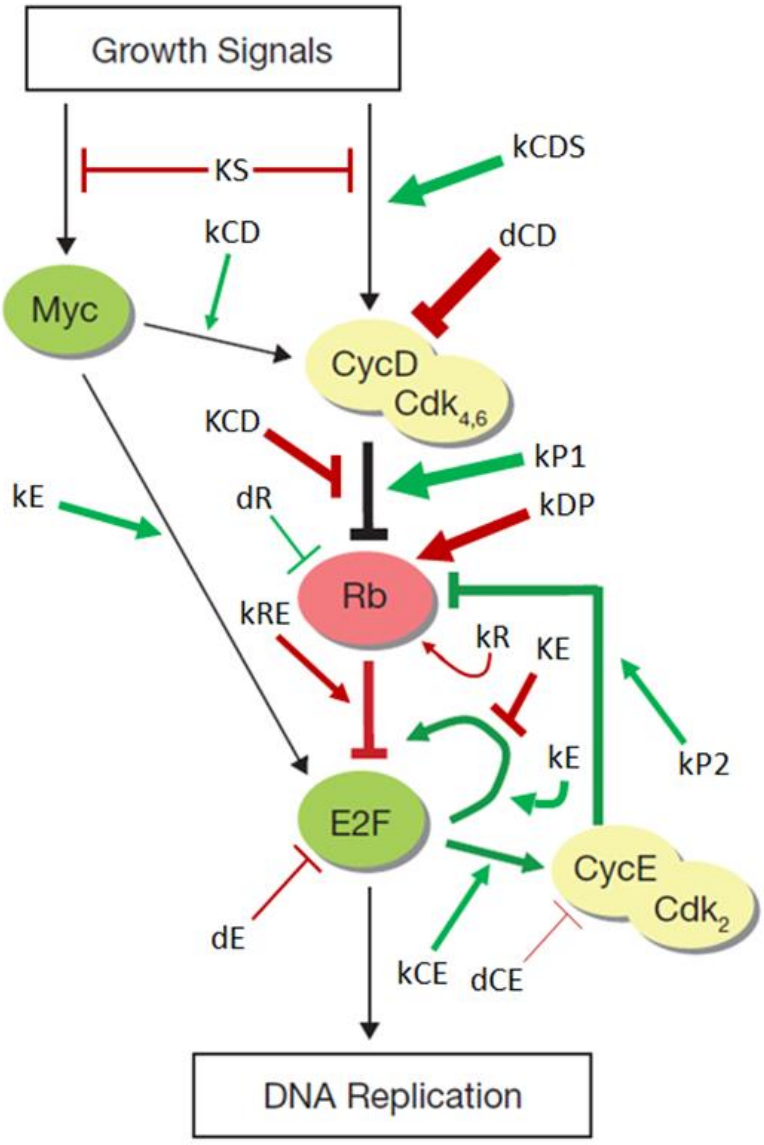

Figure 3. The simplified model from Yao et al. including the parameters involved with the Rb-E2F pathway. The thickness of the arrows are relative to parameter sensitivity, the thicker arrows represent higher sensitivity. Reproduced from Everetts 2015 (2).

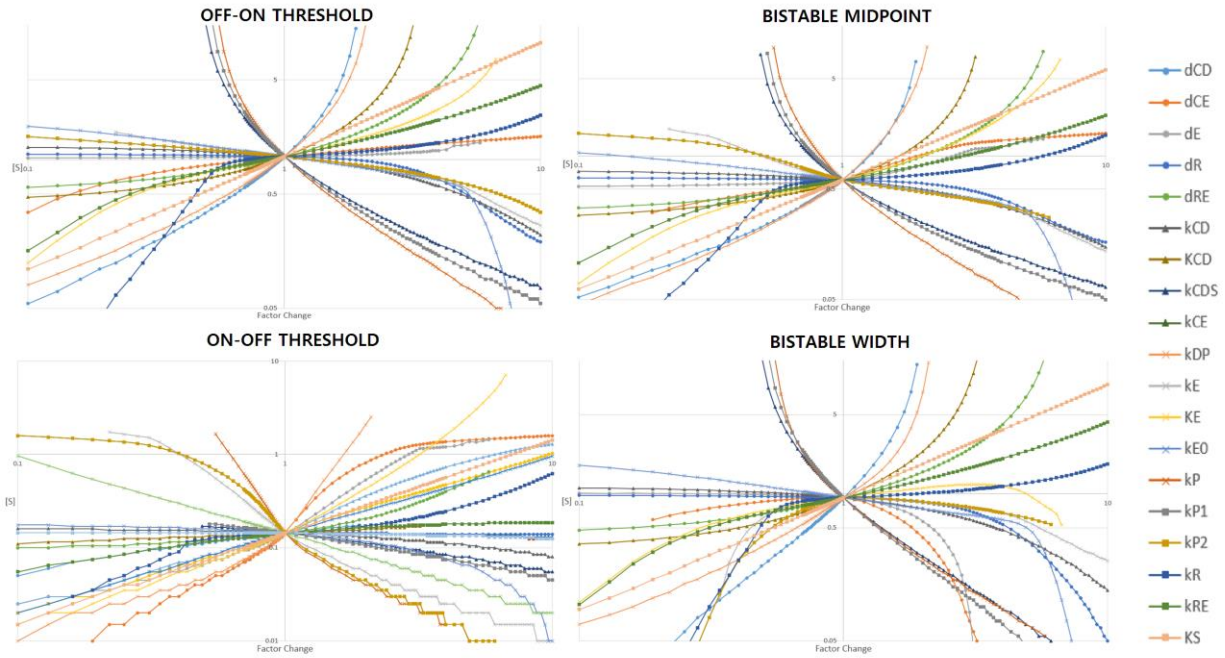

Figure 4. Spider graph analysis of the OFF-ON threshold, ON-OFF threshold, Bistable Midpoint, and Bistable Width from data acquired through COPASI simulation. Reproduced from Everetts 2015 (2).

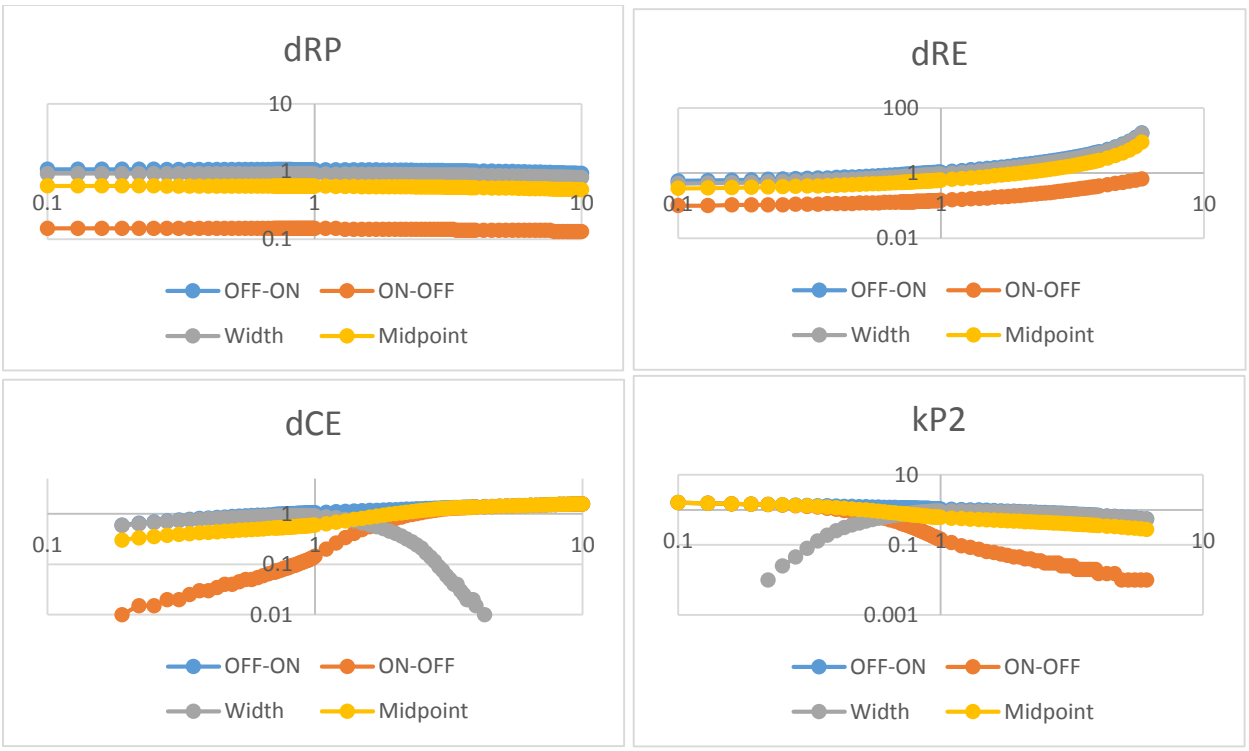

Figure 5. Representative figures of 4 different categories. Each graph was generated from the spider graph analysis performed by Everetts 2015. The four characteristics are displayed in logarithmic scale in both the X and Y axes. The factor change is relative to each characteristic and shows clear trends during decrease and increase of the single parameter. 9 categories were created from these unique behaviors.

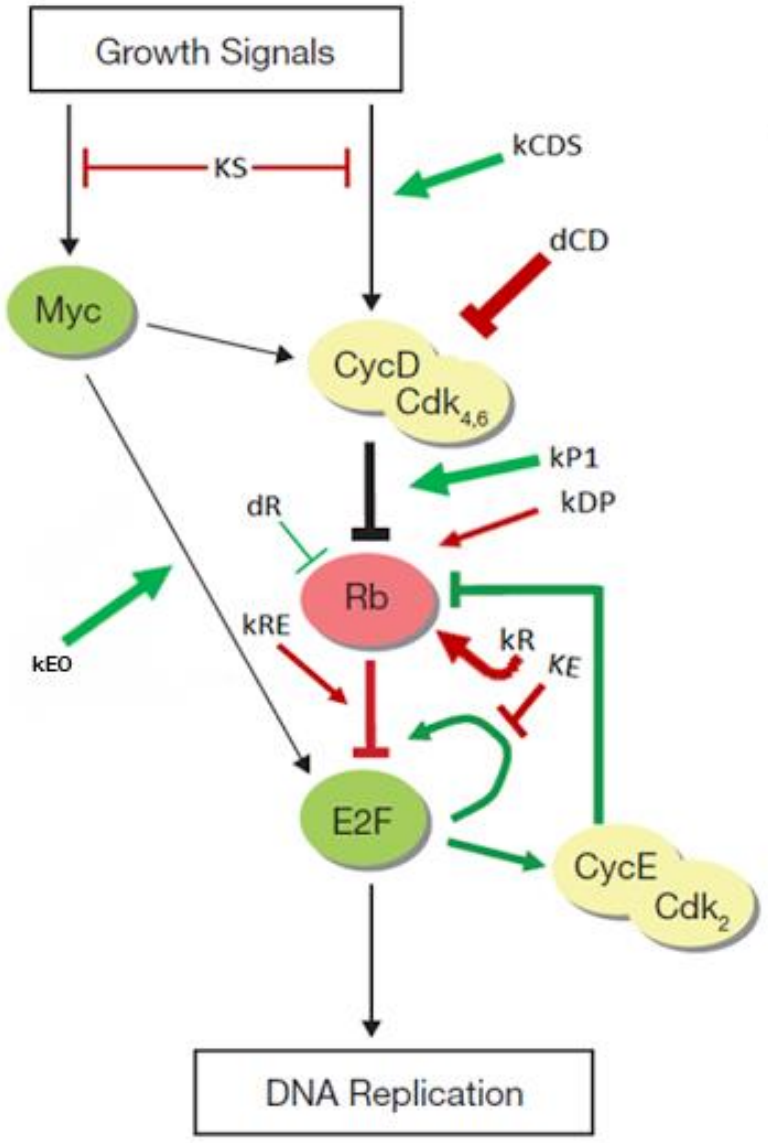

Figure 6. Schematic model diagram of the Bistable Width Decrease, Midpoint Shift Left phenotype. The color of the arrows indicate whether the parameter is increased or decreased. The thickness of the arrows represent how much decrease occurs in bistable width. The parameters involved with this phenotype are mostly focused upstream around CycD-Cdk4,6 and Rb with some parameters affecting E2F directly.

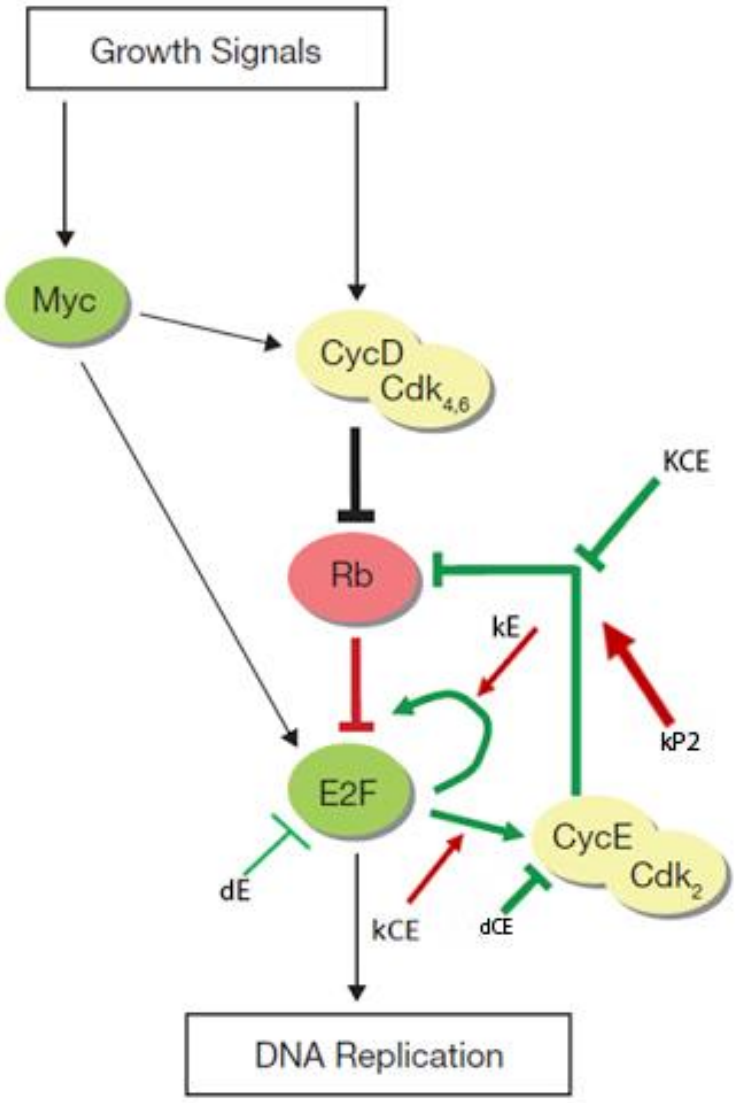

Figure 7. Schematic model diagram of the Bistable Width Decrease, Midpoint Shift Right phenotype. Constructed like Figure 6. The parameters involved with this phenotype are all affecting the downstream area of the pathway called the biomodule.

Variables	Description
S	Serum growth signals
M	Myc
E	E2F
CD	Cyclin D
CE	Cyclin E
R	Rb
RP	Phosphorylated Rb
RE	Rb-E2F Complex
Parameter	**Description**
kE0	Basal Synthesis Rate of E2F
kE	Synthesis Rate of E2F by Myc and E2F autocatalysis
kM	Synthesis rate of Myc by growth factors
kR	Constitutive synthesis rate of Rb
kb	Basal synthesis rate of E2F by Myc
kCD	Synthesis rate of Cyclin D by Myc
kCDS	Synthesis rate of Cyclin D by growth factors
kCE	Synthesis rate of Cyclin E by E2F
kP1	Phosphorylation rate of Rb by Cyclin D
kP2	Phosphorylation rate of Rb by Cyclin E
kP	Composite of both kP1 and kP2
kDP	Dephosphorylation rate of Rb
dE	Degradation rate of E2F
dM	Degradation rate of Myc
dR	Degradation rate of Rb
dRP	Degradation rate of phosphorylated Rb
dRE	Degradation rate of Rb-E2F complex
dCD	Degradation rate of Cyclin D
dCE	Degradation rate of Cyclin E
KE	Half-occupation constant of E2F
KM	Half-occupation constant of Myc
KRP	Half-occupation constant of phosphorylated Rb
KCD	Half-occupation constant of Cyclin D
KCE	Half-occupation constant of Cyclin E
KS	Half-occupation constant of growth factors

Table 1. Descriptions of the variables and parameters involved in the Rb-E2F model by Yao et al. Reproduced from Yao et al. 2008 (5).

$\frac{d[M]}{dt} = \frac{k_M[S]}{K_S + [S]} - d_M[M]$
$\frac{d[CD]}{dt} = k_E\left(\frac{M}{K_M + [M]}\right)\left(\frac{[E]}{K_E + [E]}\right) + \frac{k_b[M]}{K_M + [M]} + \frac{k_{p1}[CD][RE]}{K_{CD} + [RE]} + \frac{k_{P2}[CE][RE]}{K_{CE} + [RE]}$ $-d_E[E] - k_{RE}[R][E]$
$\frac{d[CD]}{dt} = \frac{k_{CD}[M]}{K_M + [M]} + \frac{k_{CDS}[S]}{K_S + [S]} - d_{CD}[CD]$
$\frac{d[CE]}{dt} = \frac{k_{CE}[E]}{K_E + [E]} - d_{CE}[CE]$
$\frac{d[R]}{dt} = k_R + \frac{k_{DP}[RP]}{K_{RP} + [RP]} - k_{RE}[R][E] - \frac{k_{P1}[CD][R]}{K_{CD} + [R]} - \frac{k_{P2}[CE][R]}{K_{CE} + [R]} - d_R[R]$
$\frac{d[RP]}{dt} = \frac{k_{P1}[CD][R]}{K_{CD} + [R]} + \frac{k_{P2}[CE][R]}{K_{CE} + [R]} + \frac{k_{P1}[CD][RE]}{K_{CD} + [RE]} + \frac{k_{P2}[CE][RE]}{K_{CE} + [RE]} - \frac{k_{DP}[RP]}{K_{RP} + [RP]} - d_{RP}[RP]$
$\frac{d[RE]}{dt} = k_{RE}[R][E] - \frac{k_{P1}[CD][RE]}{K_{CD} + [RE]} - \frac{k_{P2}[CE][RE]}{K_{CE} + [RE]} - d_{RE}[RE]$

Table 2. The ordinary differential equations used to construct the mathematical Rb-E2F model. Reproduced from Yao et al. 2008 (5).

Characteristics	Parameters
XL = 0 XR = 0 YL = 0 YR = 0 ML = 0 MR = 0 WL = 0 WR = 0	dRP, kb, KRP
XL = 0 XR = -1 YL = 0 YR = 0 ML = 0 MR = -1 WL = 0 WR = -1	dR, kCD, kE0
XL = 0 XR = 1 YL = -1 YR = 1 ML = 0 MR = 1 WL = 0 WR = -1	dCE, dE, dM, KCE, KM
XL = 0 XR = -1 YL = 1 YR = -1 ML = 1 MR = -1 WL = -1 WR = 1	kCE, kE, kP2
XL = 1 XR = 0 YL = 1 YR = -1 ML = 1 MR = -1 WL = -1 WR = 0	kM
XL = 1 XR = -1 YL = 0 YR = -1 ML = 1 MR = -1 WL = 1 WR = -1	kCDS, kP1
XL = 1 XR = -1 YL = 1 YR = -1 ML = 1 MR = -1 WL = 1 WR = -1	kP
XL = -1 XR = 1 YL = -1 YR = 1 ML = -1 MR = 1 WL = -1 WR = 1	dCD, dRE, kDP, kR, kRE, KCD, KS
XL = -1 XR = 1 YL = -1 YR = 1 ML = -1 MR = 1 WL = -1 WR = -1	KE

Table 3. Categorization of the parameters into groups according to their characteristics. X = OFF-ON threshold, Y = ON-OFF threshold, M = Bistable Midpoint, W = Bistable Width. L = Decrease in the parameter, R = Increase in the parameter. -1 stands for a decrease in the characteristic, 0 stands for no change, and 1 stands for an increase. There are 9 categories total.