A MITOTIC OSCILLATOR WITH A STRANGE ATTRACTOR AND DISTRIBUTIONS OF CELL CYCLE TIMES

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I. Introduction.

The concept of the existence of the cell cycle appeared shortly after the advent of light microscopy in the last century when natural scientists first described the intranuclear events involved in mitosis and cytokinesis. It was not long before significant intercellular variation in the cell generation time (the elapsed time between cell birth and the production of two daughter cells, also called the intermitotic time) was described in a variety of cell types.

The next major advance in cataloging the events of the cell cycle came when Howard and Pelc [1] utilized radioactively labelled compounds to demonstrate that DNA synthesis occupied a discrete portion of the cell cycle, and used this observation to divide the cell cycle into four discrete phases. The period between cell birth and the initiation of DNA synthesis was denoted G1, the DNA replication and mitotic phases were called S and M respectively, and the period between the completion of S phase and the initiation of mitosis was named G2. Later work [2] indicated that the G1 phase might consist of two functionally different phases in series, with the phase preceeding G1 denoted by G0. Detailed studies of many different cell lines using a variety of techniques have revealed that there is significant variation in the duration of all phases of the cell cycle [3].

Given the nature of the experimental data, it is not unnatural that most interpretations of this variability in cell phase duration were based on probabilistic considerations [4-9]. However an alternate assumption, invoking the existence of an intracellular oscillator timing the cell cycle, has also been proposed [10-14] and criticized [15]. The nature of the oscillators considered ranges from 'limit cycle' to 'relaxation' types, though the distinction is more one of degree than of type. Others, to mimic the variability of cell cycle events, have assumed that this intracellular oscillator has superimposed 'noise' [16-20]. However, this assumption begs the question of the origin of cell cycle variability and is unable to account for the observed differences in the coefficient of variation of the densities of the distributions of the various cell cycle phase durations [3].

In [21], the hypothesis that there exists a mitotic oscillator with a strange attractor timing the cell cycle was shown to lead to results identical with those derived from the purely probabilistic model presented in [9]. Interestingly, the

existence of such an oscillator seems not to have been previously considered even though it was all but explicitly postulated in [22], and all of the dynamical ingredients are present in the cell for its occurrence.

Here, a mitotic oscillator model consistent with the general formulation in [21] is used to analyze data for the duration of the cell cycle in a variety of cellular populations. Specific assumptions concerning the nature of the intracellular production of a substance or property called mitogen, and the dependence of this hypothetical oscillator on mitogen levels, allow a unique and simple deterministic model to replace a probabilistic approach.

II. The Model

In this section, the model is specified. The model rests on three hypotheses previously presented in a more general context [21].

Hypothesis 1. There exists some substance(s) (mitogen) necessary, but not sufficient, for mitosis to take place. (There is ample experimental support for this concept, as reviewed in [23]).

Consider a cell in a large population, born at time $t = 0$ with mitogen content r. Assume that mitogen levels are normalized and bounded on the closed interval $[0,2]$, and that the evolution of mitogen following birth is governed by

$$B \frac{dm}{dt} = m(2-m), \qquad m(0) = r. \tag{1}$$

where $B > 0$ is a parameter to be determined from the data. The solution of (1), denoted by $m(r,t)$, is

$$m(r,t) = 1 + \tanh \{[t - \bar{t}(r)]/B\} \tag{2}$$

where $\bar{t}(r) = (B/2) \ln [(2 - r)/r]$ and $m(r,\bar{t}) = 1$.

Hypothesis 2. There exists an oscillating intracellular variable sufficient to trigger mitosis once it exceeds a threshold value.

More precisely, let $x(t)$ denote the value of this variable at time t, and t_n, $n = 0,1,\ldots$, denote the times at which $x(t)$ attains a relative maximum. If $x_n = x(t_n)$ are the values of these relative maxima, then it is assumed that

$$x_{n+1} = m(t_n)S(x_n) \tag{3}$$

where $S:[0,1] \to [0,1]$ is a Rényi transformation, and that mitosis takes place whenever $x_n > 1$. (A mapping $S:[0,1] \to [0,1]$ which satisfies:

i) There exists a partition $0 = a_0 < a_1 < \ldots < a_p = 1$ of $[0,1]$ such that for each integer i, $i = 1,\ldots,p$, the restriction S_i of S to the open interval (a_{i-1}, a_i) can be extended as a C^2 function to $[a_{i-1}, a_i]$;

ii) $S_i([a_{i-1}, a_i]) = [0,1]$, $i = 1,\ldots, p$; and

iii) $\inf_{(a_{i-1},a_i)} |S'(x)| > 1 \quad i = 1,\ldots,p$

is called a Rényi transformation, see [24]).

As shown in [21], the consequence of this hypothesis in conjunction with a theorem of Lasota and Yorke [25] is that once the mitogen level exceeds the threshold value of 1, the mitotic rate is $S(m-1)$, where $S > 0$ is constant. Therefore, if $\alpha(r,t)$ denotes the fraction of cells born with mitogen level r that have not divided by time t, then

$$\alpha(r,t) = \begin{cases} 1 & 0 < t < \bar{t}(r) \\ \exp\left[-S\int_{\bar{t}}^{t} [m(r,y)-1]\, dy\right] & \bar{t}(r) \leq t. \end{cases} \tag{4}$$

Note that $-\alpha_t(r,t) = -\partial\alpha(r,t)/\partial t = S[m(r,t)-1]\alpha(r,t)$ is the density function for the distribution of intermitotic (generation) times in this population of cells having initial mitogen levels r.

Using the solution for $m(r,t)$ given in equation 2, $\alpha(r,t)$ assumes the explicit form

$$\alpha(r,t) = \begin{cases} 1 & 0 < t < \bar{t}(r) \\ \{\cosh[t-\bar{t}(r)]/B\}^{-SB} & \bar{t}(r) \leq t. \end{cases} \tag{5}$$

Generally, the initial mitogen level r in a population of cells will be distributed on $[0,1)$ with a density $f(r)$, so $\alpha(t)$ for the entire population is given by

$$\alpha(t) = \int_0^1 \alpha(r,t) f(r) dr. \tag{6}$$

Finally, the density function for the distribution of generation times for the entire

population is given by

$$\psi(t) = -\int_0^1 \alpha_t(r,t)f(r)dr. \tag{7}$$

To complete the specification of this model, the distribution f(r) of initial mitogen levels in the population of cells is required. This results in the third and final hypothesis of the model:

Hypothesis 3. Each sister cell receives exactly one-half of the mitogen present in the mother cell at mitosis.

As shown in [9], from this and the previous hypotheses there is a globally asymptotically stable distribution of mitogen f in the population of cells given by

$$f(r) = \begin{cases} 0 & 0 < r < 1/2 \\ 2q(2r)\exp\left[\int_1^{2r} q(z)dz\right] & 1/2 \leqslant r < 1. \end{cases} \tag{8}$$

where $q(y) = SB(y-1)/y(y-2)$. Carrying out the integration in equation 8 gives

$$f(r) = \begin{cases} 0 & 0 < r < 1/2 \\ 2SB\,\dfrac{2r-1}{4r(1-r)}\,[4r(1-r)]^{\frac{1}{2}SB} & 1/2 \leqslant r < 1. \end{cases} \tag{9}$$

Another statistic, widely used by cell kineticists in characterizing populations of renewing cells, is the fraction of sibling cell pairs whose intermitotic times differ by at least a time t. This fraction is denoted by $\beta(t)$. The derivation of $\beta(t)$ given in [9] for this hypothetical cellular population leads directly to

$$\beta(t) = -2\int_0^\infty \int_0^1 \alpha_t(r,x)\alpha(r,x+t)f(r)dxdr,$$

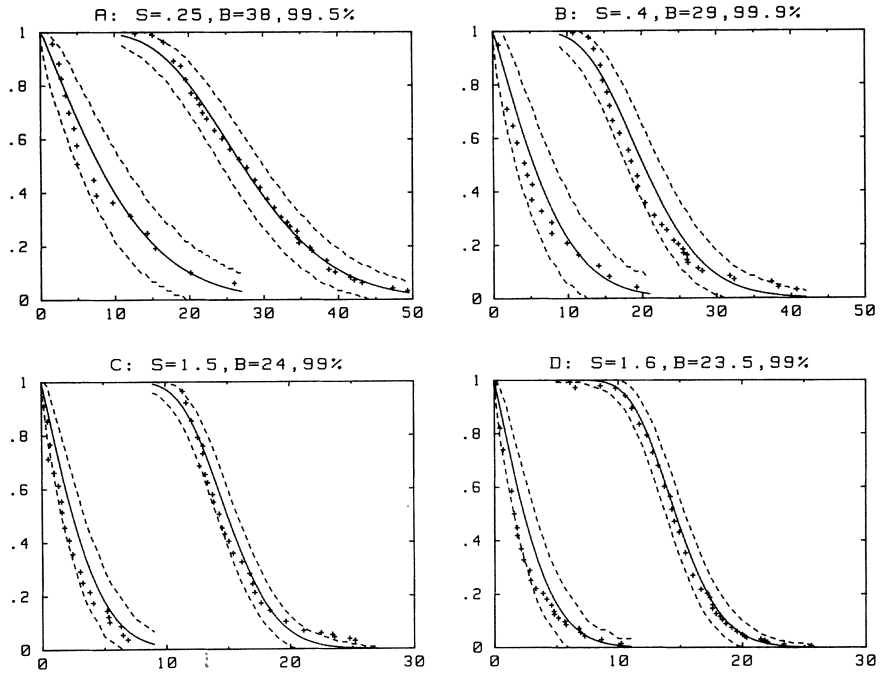

Figure 1. Correspondence between model predictions and data from SV3T3 cells grown at four different concentrations of fetal calf serum. A: Serum = 0.05%; B: 0.25%; C: 1.0%, and D: 10.0%. Data from [27]. In each panel of this and succeeding figures, the vertical axis is α or β and the horizontal axis is cell age in hours, the data points are indicated by (+), and the best fitting $\alpha(t)$ and $\beta(t)$ curves predicted by the model, based on the indicated parameter values, are represented by solid lines. The dashed (---) lines bounding the predicted $\alpha(t)$ and $\beta(t)$ curves give the confidence intervals corresponding to the percentages indicated at the top of each panel. [A confidence interval value of 99% means that if the data can be described by $\alpha(t)$, then the probability that, at a given t_j, the observed value $\alpha(t_j)$ will be either above or below the confidence interval is 1% or less.]

which, under the assumptions of this paper, takes the form

$$\beta(t) = 2 \int_0^\infty x \tanh(x/B) \{\cosh(x/B) \cosh [(x+t)/B]\}^{-SB} dx. \qquad (10)$$

III. Analysis of the Data.

Equations 5, 6, and 9 and 10, respectively specify the functions $\alpha(t)$ and $\beta(t)$ and involve only two parameters, S and B. We examined the ability of this model of the cell cycle to account for 18 existing published data sets $\{t_j, \alpha(t_j)\}$,

$\{t_j, \beta(t_j)\}$, though here we present only some of the results of our analysis.

We digitized and stored the data sets $\{t_j,\alpha(t_j)\}$, $\{t_j,\beta(t_j)\}$, and then determined the parameter values that gave the best fit to the data using nonlinear regression techniques [Santavy, in preparation]. We first determined the values of S and B that gave the best fit to the $\{t_j, \alpha(t_j)\}$ data, and then compared the resulting predicted $\beta(t)$ with the actual $\{t_j,\beta(t_j)\}$ data set.

The results of this procedure are illustrated in Figure 1 for SV3T3 cells grown at four different concentrations of fetal calf serum. Note that with increasing serum levels, there is a progressive elevation in the parameter S and a concomitant fall in the parameter B.

The same procedure was followed in an examination of the ability of this oscillator model of the cell cycle to account for data from eight other cell lines as shown in Figure 2.

For the data analyzed in Figures 1 and 2, as well as other cases that we have not presented, we are able to achieve a more than satisfactory correspondence between the model predictions and the $\{t_j,\alpha(t_j)\}$ data. However, the predicted $\beta(t)$ often deviates significantly above the data, i.e. sister cell pairs have differences in intermitotic times significantly less than predicted based on the population response.

Noting this, we followed two different data fitting procedures, illustrated for the BHK 21 cells of Figure 2 (reproduced in Figure 3A). First, the parameters S and B were determined to give the best fit to the $\{t_j,\beta(t_j)\}$, and then the $\alpha(t)$ curve was predicted (Figure 3B). Second, S and B were determined to give the best fit to both the $\{t_j,\alpha(t_j)\}$ and the $\{t_j,\beta(t_j)\}$ data (Figure 3C). It is clear that neither procedure gives a satisfactory fit to the data.

One might question whether the failure of this model to account for the existing sets of data is due to the improper choice of the assumed dynamics of mitogen production (Equation 1). However, it is easy to show that this is not the case. Consider the following. Assume that a population of N cells is composed of m different subtypes, with each different subtype characterized by a different mitotic rate. Let $h_i(t)dt$ be the fraction of the ith cell type with intermitotic time T_i such that $t < T_i < t+dt$ so $h_i(t)$ is the mitotic rate for the ith type of cell. Further take $h_i(t) = 0$, $0 < t \leq t_1$, $h_i(t) \leq H_i$ for $t_1 < t \leq t_2$, and $h_i(t) = H_i$ for $t_2 < t$. Then the mitotic rate for the entire cellular population satisfies $h(t) \leq H = \sum w_i H_i$ for $t_1 < t \leq t_2$ and $h(t) = H$ for $t_2 < t$, where w_i is the fraction of the total population comprised of the ith cell type. Thus, the logarithmic slope of the $\alpha(t)$ curve for the entire population will be constant and equal to -H for sufficiently large t. Now consider sister cell pairs drawn from this same population. Then, the mitotic rate for a cell of type i at time t+X whose sister divided at time X is simply $h_i(t+X)$ which, for $t_2-t_1 < t$, is simply H_i. A straightforward argument then shows that for sufficiently large times t the

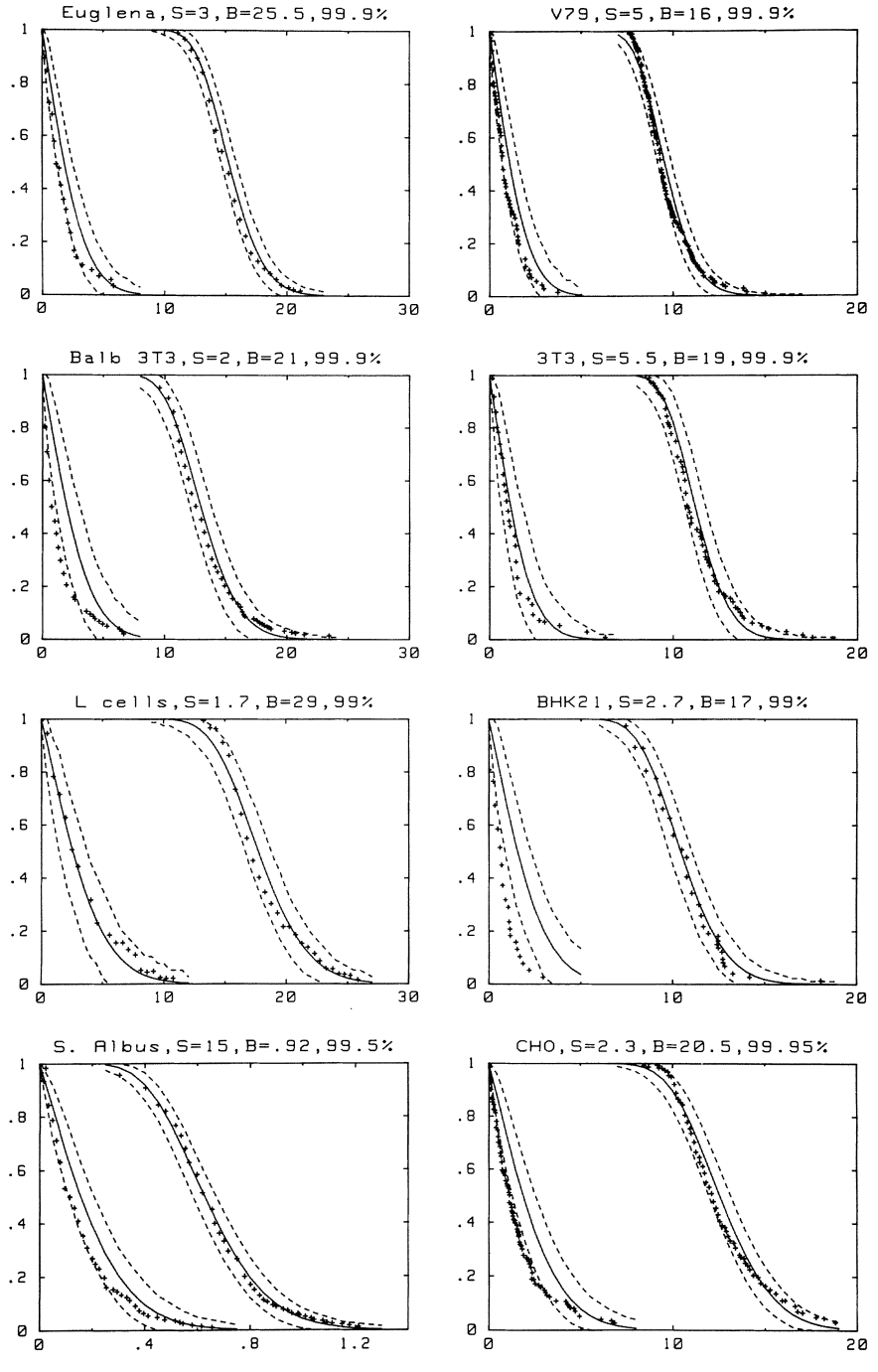

Figure 2. The results of determining the best model fit to eight sets of data. Data sources: Euglena gracilis [26], Balb 3T3 [27], L cells [28], S. Albus [29], V79 [30], 3T3 [31], BHK21 [32], and CHO [31].

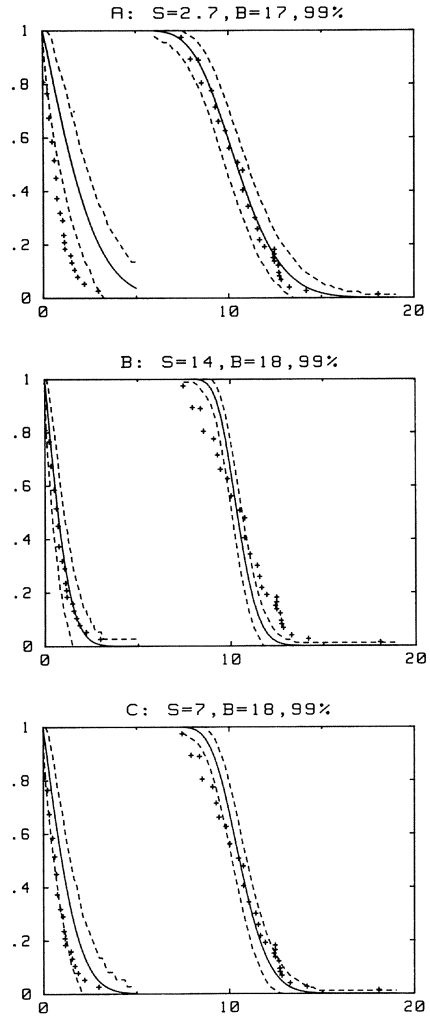

Figure 3. The results of three different procedures to fit data from BHK 21 cells [27]. A: The result of fitting the α data and predicting β, reproduced from Figure 2; B: The fit to β and the predicted α; and C: The consequence of fitting the α and β data together.

logarithmic slope of the $\beta(t)$ function must be identical to -H. Thus, an improper choice of the mitogen dynamics in equation 1 cannot be the source of the discrepancy between the observed and predicted behaviour of sister cell pair intermitotic times.

To have more control over t_1 and t_2 than was available in our formulation of the model, we introduced a third parameter D such that the mitotic rate is given by $S[(m(r,t)-1)/D]$ for $1 \leq m(r,t) < 1+D$ and is just S for $m(r,t) \geq 1+D$. With this

modification it was possible to achieve equal predicted logarithmic slopes for both $\alpha(t)$ and $\beta(t)$ at smaller values of t and not just in the limiting ($t\to\infty$) case. However, this modification was insufficient to give a satisfactory agreement between the model predictions and the existing data, for the $\beta(t)$ data once again displayed a significantly greater (in absolute value) slope than predicted from the model.

IV. Discussion.

Of the three hypotheses used in the development of this model, the major mathematical and biological questions revolve around Hypothesis 3.

First, note that probabilistic formulations of cell cycle models are mathematically identical with a formulation based on the existence of a mitotic oscillator with the characteristics detailed in Hypothesis 3 (compare [9] and [21]). However, there are significant and profound differences in the interpretation of the underlying biology within the context of the two formulations.

Second, is there any oscillating dynamical or semi-dynamical system with successive maxima determined by a Rényi transformation? Any continuous time system with this property must, by necessity, be of dimension 3 or greater. Though there is no analytic proof of the existence of such systems at this time, there is good numerical evidence for their existence as shown in [33] and [34] from an analysis of the Lorenz equations.

Third, do intracellular oscillations exist? Again, the answer is yes. For example, Rapp [35] has cataloged literally hundreds of reported oscillations of cellular events occurring on relatively short time scales. More recently, a variety of investigators (see Lloyd and Edwards [36]) have noted oscillations in cellular respiration, a variety of enzymes, the adenine nucleotides, and ATPase activity to name but a few. Finally, Klevecz (see [13,37]) has collected data from a number of cell lines which he interprets as evidence for the existence of a quantal oscillator clocking the cell cycle with a period of the order of 4 hours.

Fourth, do there exist known intracellular biochemical control pathways capable of generating irregular oscillations? Biochemical control loops within the cycling cell are numerous, richly interconnected, and nonlinear. Further, many display mixed positive/negative feedback with or without significant time delays in their feedback pathways [38-40]. As has been repeatedly shown numerically [41,42] these are exactly the conditions under which strange attractors and the attendent properties required by the theory developed in [21] may be encountered. Two examples immediately come to mind.

The general scheme for the production of repressible enzumes put forward in Tyson and Othmer [38] contains multiple nonlinearities as well as time delays required for transcription and translation. A subsequent application of this model to the tryptophan operon in E. coli [40] experimentally demonstrated the instability of this gene control network and the existence of oscillations.

A second example involves the biochemical control networks for purine and pyrimidine synthesis which supply necessary precursors for DNA replication and the initiation and maintenance of transcription. These networks are highly interconnected with multiple positive, negative, and mixed positive-negative feedback loops. Preliminary simulations of the dynamics of this system [Mackey, unpublished] with known or estimated parameter values and feedback functions indicate that there are large regions of parameter space in which steady states are unstable and highly irregular oscillations may occur.

All of the failures, noted in the previous section, to obtain better agreement between the existing data and the one dimensional oscillator model formulated here may be traced to the fact that the oft claimed equality of the limiting logarithmic slopes of the $\alpha(t)$ and $\beta(t)$ data is only approximate. This experimental observation thus disqualifies all one dimensional models (either probabilistic or deterministic) for the statistics of the cell cycle, and suggests that a multi-dimensional model would be more appropriate. However, with the introduction of a multi-dimensional mitotic model we would also be in the position of not being able to check the validity of such a model based only on existing $\alpha(t)$ and $\beta(t)$ data. For example, a two-dimensional model will be able to easily fit both the $\alpha(t)$ and $\beta(t)$ data (even if they are independent as assumed in the Brooks two random transition model [31]), but the validation of such an extended model would require the availability of a third statistic in addition to $\alpha(t)$ and $\beta(t)$.

Of the known events associated with the cell cycle, the two most recognizable are mitosis and DNA synthesis. We feel that the observations cited above concerning the potential behaviour of the biochemical control networks involved in the synthesis of DNA precursors, along with the failure of a single threshold oscillator model as presented here to account for the sister-sister intermitotic time data, offer a strong motivation for expanding the model to include two oscillators -- one for the initiation of DNA synthesis, and the second for the initiation of mitosis.

There are a number of attractive aspects to the hypothesis that variability within the cell cycle may arise from the operation of an oscillator with a strange attractor.

First, if the present formulation were extended to a higher dimensional threshold crossing oscillator model it offers the possibility to understand the deterministic origin of the variability found within specific phases of the cell cycle [3]. Second, if amplitude characteristics of the oscillator and/or threshold levels are responsive to a variety of extracellular factors (eg., the serum effects shown in Figure 1) then the model offers a different interpretation of the current view of the GO state. Third, if intracellular oscillators are assumed able to cross threshold for DNA synthesis and to occasionally miss a mitotic threshold, then the hypothesis offers a qualitative explanation for the existence of polyploid cells in normal tissue. An alteration of oscillator and/or threshold characteristics in

transformed cells could explain the increased frequency of polyploid cells in abnormal tissues. A similar explanation would suffice to explain the existence of reduction divisions. Fourth, irregular oscillations in the pools of DNA precursors in conjunction with a threshold for the initiation of DNA synthesis could serve to explain data that have often been interpreted to indicate that DNA synthesis may be intermittent. Finally, the model would have sufficient flexibility to explain the fact that DNA synthesis and mitosis may not always occur in a sequential fashion as noted, for example, in rapidly growing bacteria.

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