



GENE EXPRESSION DURING THE CELL CYCLE: OBFUSCATION OF ORIGINAL CELL-CYCLE GENE EXPRESSION DATA BY NORMALIZATION

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ABSTRACT

Normalization of raw data on gene expression during the cell cycle obscures the original experimental data and makes it appear as if all genes have the same numerical level of cyclical expression. Results that would not support gene expression because of minimal variations thus appear, after normalization, to be stronger than they actually are. Consideration of the effect of normalization raises critical questions about many experiments on the cell cycle dependent variation of gene expression—that is, proposed cyclical changes in mRNA content—during the cell cycle.

Keywords: Cell-cycle, Normalization, Gene expression, mRNA synthesis.

Contribution/ Originality

This paper demonstrates that normalization makes minimal variations appear much stronger than they are in the raw data, and thus one should be skeptical of some published results proposing cell-cycle specific gene expression.

1. ALTERNATIVE MODELS OF CELL-CYCLE PROGRESSION

The current, consensus, and widely-accepted “Standard Model” of the cell cycle envisions that passage through the cell cycle involves the sequential expression of numerous genes at different times during the cell cycle [1-8]. Support for the Standard Model stems primarily from experimental data measuring the amount of different mRNA’s during the cell cycle of cells that are proposed to be synchronously moving through the cell cycle. The Standard Model has received additional theoretical support from the “just in time” model proposing that time-dependent gene expression is important and logical because gene products are produced at times when these gene products are needed [2].

An alternative view of passage through the eukaryotic cell cycle, the “Continuum Model”, proposes that there are no or few genes whose expression varies during the division cycle [9-14]. Support for the Continuum Model has been based on critiques of the Standard Model showing

that experimental data upon which the standard model rests is flawed [15, 16], and even more important, that even if mRNA varied during the division cycle the variation of mRNA has minimal to non-existent effects on protein content during the cell cycle [17]. In addition, there is experimental support for the invariance of gene expression during the cell cycle [18].

It is possible, and probable, that data showing no or minimal variation in gene expression during the division cycle do not appear in the literature as these results are sometimes considered “negative” data and are thus not worthy of publication. Data inconsistent with the Standard Model may not appear in the literature due to this bias against presenting data not showing “positive” results.

The most devastating critique of the Standard Model comes from the logical problems with the proposed existence of a large number of genes giving varying cell-cycle expression patterns, problems that have not been considered and that subvert the current, consensus viewpoint [11]. The logical critique points out that variation of gene expression during the cell cycle requires the cycle-specific appearance of some activation element and then the cycle-specific appearance of some element that removes the product of gene expression. These activating and inactivating elements themselves require the appearance of cycle-specific cellular elements, *ad infinitum*. Until this logical critique—referred to as the Russian Doll Problem [11]—is disposed of or answered, one must be wary of accepting the tenets of the Standard Model.

The data supporting the current, consensus, dominant, widely-cited, and well-recognized Standard Model stem mostly from the most used and flawed approach to cell-cycle analysis, the use of whole-culture synchronization methods that do not synchronize cells [13, 19-29]. The popular methods used for “synchronizing” cells are not criticized here because they synchronize cells poorly, or only occasionally, or that synchrony does not last, but because these batch or whole-culture inhibition methods do not synchronize cells at all. Whole-culture inhibition methods where cells are growth inhibited and where it is proposed that the cells are synchronized upon release of inhibition do not synchronize cells. These methods have been shown to not synchronize cells by both theoretical analysis [19, 23, 24] and by numerous experimental analyses [21, 27-29]. Even publications that propose that methods such as serum starvation or thymidine inhibition can synchronize cells, these papers often present data that these methods do not synchronize cells [1] when considered in the light of clearly defined criteria for synchronization [19].

2. CRITICAL ANALYSIS OF NORMALIZATION OF CELL-CYCLE EXPRESSION DATA

The purpose of this paper is to critique the normalization of cell-cycle related gene expression data that has supported the Standard Model of gene expression during the cell cycle. I illustrate how normalization of raw data obscures the original data, and thus leads to the publication of graphs that make minimal variations appear much stronger than they actually are.

The common approach to normalization is to replot the original data (not usually shown) so that the final graph shows the data from different genes on the same scale. For example, normalization could produce sine waves with a “mean of 1 and an amplitude of 2”.

Although there are many examples of the normalization of results, a particularly good example is found in a paper by Orlando, et al. [5].

An illustration of the normalization process and problem is presented in Fig. 1. Fig. 1A plots six idealized sine waves of differing amplitudes and different peak times during the cell cycle. Figs. 1 C, D, E show that if a shallow sine wave is plotted with appropriate values in the ordinate, a shallow or essentially flat line can appear quite sinusoidal. Fig. 1B presents the results following the normalization of the lines in Fig. 1A to show that each of the lines is a sine wave of similar amplitude. The results in Fig. 1 speak for themselves. Sine waves of different amplitudes, when normalized show equivalent strengths of sinusoidal variation (Fig. 1B). Yet the original data (such as found in Fig. 1A) have graphs that are of very small variations

The graphs in Fig. 1 illustrate that normalization can make data that would not pass muster indicating variation in gene expression during the cell cycle appear much stronger. For example, the flattest line in Fig. 1A has data that varies over only 6%, and that is for the maximum value and the minimum values. Experimental variation is generally much greater than 6% and thus the original data could very well be due to minor experimental variations. Normalization obscures data that would not be accepted as significant and makes data palatable when it would be discarded.

I hope that the field can now re-evaluate previous data in the light of the normalization problem (as well as the synchronization problems and the lack of reproducibility problems [15] and reconsider the entire status of the current belief in the Standard Model.

3. THE CONTINUUM MODEL

The Continuum Model stands in stark contrast to the Standard Model as it proposes that no genes are expressed at particular times during the cell cycle and that mass increases during the division cycle in a simple, continuous exponential manner with no time varying pattern of gene expression. Decreases in specific proteins at particular times are due only to their destruction at particular times during the division cycle, primarily the mitotic phase [18, 30]. This proposal avoids the problems of the Russian Doll Problem [18, 30].

Furthermore, the Continuum Model has predicted that growth of cells will be simple and exponential during the division cycle, a prediction that has been recently born out by a reconsideration of the growth of *Schizosaccharomyces pombe* during the division cycle [31].

To the many critiques of the Standard Model—that methods used to synchronize cells do not synchronize cells [13, 19-29], that data in support of the standard model have been shown to be not reproducible [15, 16], that gene expression variations cannot have any significant affect on passage through the cell cycle [17], that logical problems with the Standard Model have not been

addressed [11]—I now add a critique of the use of normalization to present data on gene expression during the division cycle.

4. FIGURES

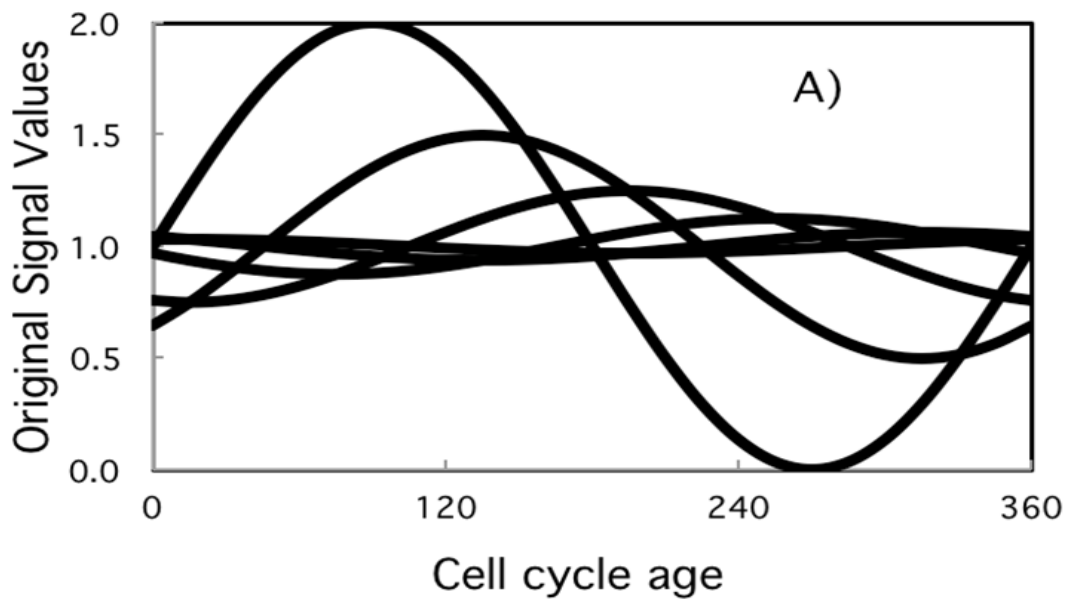
Fig. 1. A Six sine waves of different amplitudes are plotted. The graph values have an average of 1.0, and the max/min values of the six graphs are: 2.000/0.000; 1.500/0.500; 1.250/0.750; 1.125/0.875; 1.063/0.938; and 1.031/0.969. These six graphs were generated by taking a sine wave and adding different constant values each of the values and then making the values have an average value of 1.0. The larger the value added the flatter the sine wave. The peaks for each graph are placed at different points during the cell cycle. B The data from panel A are normalized to a peak of 2.000 and minimum of 0.000. (C, D, E) Three patterns from panel A are plotted with different ordinate scales to show that when expanded the graphs that are shallow in panel A are now indicative of a sinusoidal pattern.

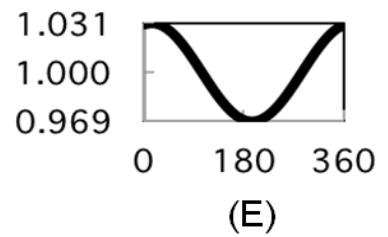
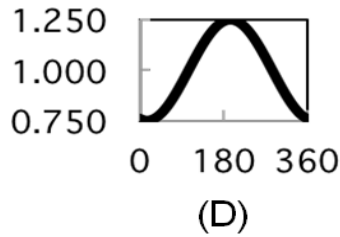
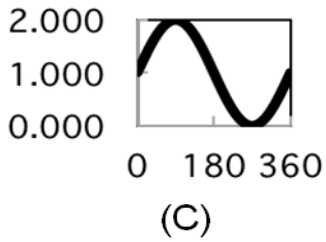
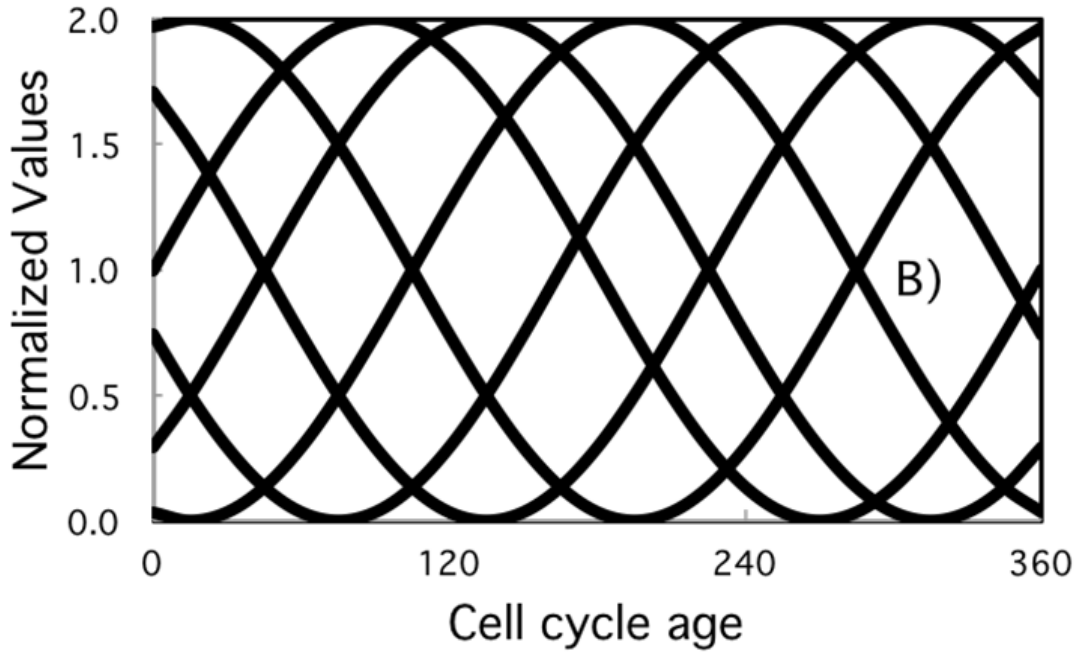
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