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Critical Review

On a Heuristic Point of View Concerning the Expression of Numerous Genes During the Cell Cycle

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Summary

The current model of the eukaryotic cell cycle proposes that numerous genes are expressed at different times during the cell cycle. The existence of myriad control points for gene expression leads to theoretical and logical problems for cell cycle control. Each expressed gene requires a control element to appear in a cell-cycle specific manner; this control element requires another control element and so on, *ad infinitum*. There are also experimental problems with the current model based on ineffective synchronization methods and problems with microarray measurements of mRNA. Equally important, the efficacy of mRNA variation in affecting changes in protein content is negligible. An alternative view of the cell cycle proposes cycle-independent, invariant accumulation of mRNA during the cell cycle with decreases of specific proteins occurring only during the mitotic period of the cell cycle.
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INTRODUCTION

The passage of a cell through the cell cycle is currently believed to involve a large number of changes in gene expression as the cell passes from birth by division to the ultimate mitotic cycle. The current or standard model of the cell cycle has a central problem due to the large number of genes whose expression changes at various times during the cell cycle. I will present an analysis that leads to a different view of the passage of a cell through the cell cycle. Because these ideas are in large part theoretical, it is important to consider different ways of looking at theoretical proposals.

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In a discussion of the coding problem, Francis Crick (*1*) had a short section entitled "On the Place of Theory." His ideas are worth considering anew. Here, I present the relevant part in full. [Although the section cited deals with the particular problem of the nature of the genetic code, the essential ideas are widely applicable.]

> It does not seem to be appreciated that theoretical work is often of two rather distinct types. There is first the deduction from experiment: the weighing of the data and the reasoned assessment of, say, the evidence that a particular codon represents a particular amino acid. This I would call interpretation, and it needs to be done critically.
>
> Second, we have theory proper. This may take several forms: for example, Wall's demonstration that a partially overlapping code is not yet eliminated; or Woese's attempt to deduce the whole structure of the code from only part of it. *These theories may not be correct but they are both sensible and useful, in that they enable us to tighten up our logic and make us scrutinize the experimental evidence to some purpose*. Moreover, even if Woese's code is wrong, his careful exploration of its consequences may enable us to see something about the general character of the genetic code. But, most important of all, these ideas are not merely useful, they are novel. *If their authors had not suggested them, they might not have occurred to many people working on the problem.* [Italics added].

It is in the category of the second type of theory that I raise critical questions regarding the control of the passage of cells through the cell cycle. As noted in the first italicized section above, it may be that the ideas I propose here are not correct. Nevertheless, I suggest that the ideas presented below raise important points that have not been generally or widely considered.

I question the widely accepted proposal, based on theoretical ideas (*2*) and numerous published experiments, that a large number of genes are preferentially expressed at particular times during the cell cycle. Equally important, these peaks of gene expression appear to occur continuously during the division

cycle with peaks of gene expression occurring at many different times during the cell cycle. This continuity of gene expression implies that genes are not expressed together in groups at a few particular times during the cell cycle but are expressed at numerous appropriate times during the cell cycle.

This article raises questions that may have occurred to some researchers on cell-cycle specific gene expression but which do not appear to have been considered in any detail. In addition to the critical questions raised regarding the fundamental proposal of numerous patterns of cycle-specific gene expression, this article will also raise questions regarding the efficacy of cell-cycle variations in mRNA content in regulating variations in protein content.

Before presenting the critique of the current, dominant, and consensus model of passage through the cell cycle, a brief review of the experimental data on gene expression during the cell cycle is offered.

EXPERIMENTAL STUDIES OF GENE EXPRESSION DURING THE CELL CYCLE

There have been a number of studies of global gene expression during the eukaryotic division cycle using microarrays to analyze mRNA content as a function of cell-cycle age. Following the studies of mRNA content in *S. cerevisiae* (*3, 4*), different groups have studied such diverse eukaryotic cells as primary human fibroblasts (*5*), HeLa cells (*6, 7*), *Arabidopsis thaliana* (*8*), *S. pombe* (*9–12*), and *Candida albicans* (*13*) as well as the prokaryote *Caulobacter crescentus* (*14*). The general result emanating from these studies is the proposal that numerous genes—as measured by mRNA content—are expressed in a cell-cycle-specific manner.

More important, these numerous patterns of proposed cyclical gene expression occur in a continuous manner (*4, 15*) so that there must be controls regulating the timing of gene expression at numerous points throughout the cell cycle. If cyclical gene expressions were grouped, with the groups each containing a number of gene products being expressed at approximately the same time, one could then postulate a small number of controls for these groups. The problem becomes more difficult when numerous genes are expressed at many different times during the cell cycle. In this case, numerous control systems must be postulated.

In addition to mRNA variations, there are also variations in protein content during the cell cycle. Proteins have been classified by the cell-cycle age or time or cell-cycle phase at which protein content peaks or is rapidly synthesized (*16–20*). In particular, it has been proposed that some proteins have a peak in content during the G1 phase or the S phase of the cell cycle. A recent review of the breakdown of proteins during the cell cycle has concluded that many proteins decay specifically during mitosis (*21*).

The question arises as to how myriad cyclical gene expression patterns and protein variations—widely believed to be important in regulating cell passage through the cell cycle—are regulated during the cell cycle.

PROBLEM OF GENE EXPRESSION—mRNA SYNTHESIS—AT A SPECIFIC TIME DURING THE CELL CYCLE

Consider a gene whose expression (*i.e.*, mRNA content) peaks at some particular cell-cycle age or phase. Assume that the increase in mRNA content is due to an increase in the rate of mRNA synthesis at a particular cell age. For this change to occur, some cellular element that controls that gene's rate of mRNA synthesis must appear; call this "control element #1." The postulation of a specific control element arises from the general idea that the synthesis of mRNA does not vary without some external influence; that is, the rate of mRNA synthesis does not vary without some intervention by an external element.

How is control element #1 regulated? There are two aspects of this control system that must be considered—the cycle-specific appearance or activation of control element #1 and its disappearance—after it has performed its function. To explain mRNA variation one must postulate some increase in control element #1 (assuming it is a positive control element) to stimulate mRNA synthesis. Control element #1 is presumably regulated by "control element #2" which is regulated by "control element #3." Continuing this process we could imagine control elements #4, #5, and so on, *ad infinitum*.

The cascade described above must be mirrored by an inverse cascade where the control elements lead to the disappearance of the activating element or elements. The removal of the activating elements after they have performed their function is necessary so that gene expression is not continuously high during the cell cycle. If the activating elements were not removed; they would persist into the next cell cycle and interfere with the cycle-specific expression of mRNA. The control elements described above (*i.e.*, 1, 2, 3, 4, 5**...**) that increase the rate of mRNA synthesis must be removed by some inhibiting or degrading elements. Control #1 is destroyed (at a particular time after the peak of RNA synthesis) by control element #–1, control element #2 is destroyed by control element #–2, and so on. The degrading elements must be gene (mRNA) specific; otherwise, there would be an inappropriate degradation of control elements, presumably proteins.

If control of gene expression is determined by variation in the decay of mRNA during the cell cycle instead of induction of synthesis, one must postulate a cycle-specific variation in production of cellular elements controlling mRNA degradation. These mRNA-degradation elements must be mRNA specific; otherwise, there would be inappropriate changes in mRNA content during the cell cycle. Similar to the synthesis-promoting elements, there would be a sequence of control elements affecting decay at various times during the cell cycle. There would also have to be control elements that degrade, or inhibit, the mRNA degrading systems. Therefore, the problem of infinite

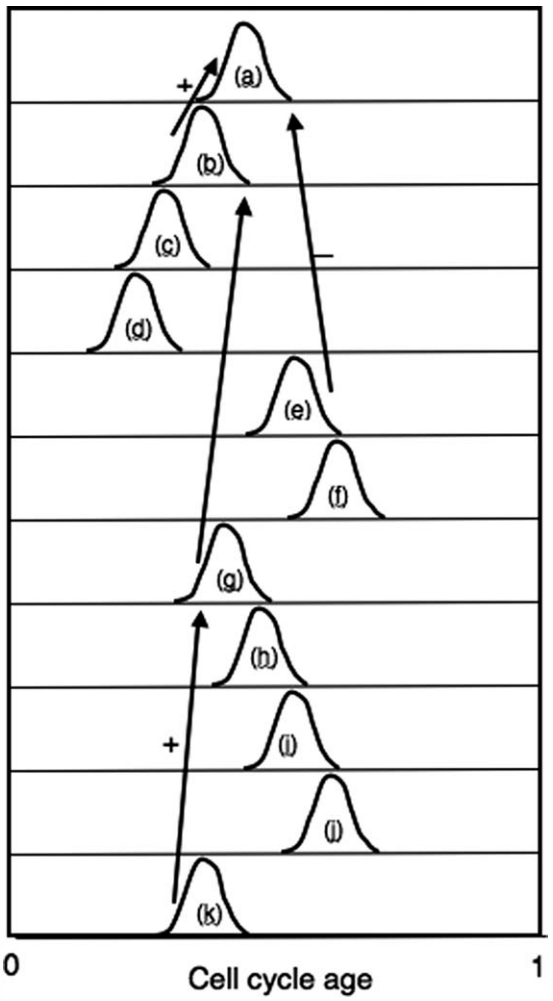

Figure 1. Schematic illustration of the infinite regression problem. Consider that a particular cellular element (a) appears at a particular time in the middle of the cell cycle. In order for this to happen, one may postulate another element (b) that stimulates the appearance of element (a). But control element (b) requires its own initiator, (c), and this in turn is controlled by element (d) and so on. The cellular element under consideration (a) is also controlled in a negative manner by element (e) which causes (a) to decay. The appearance of (e) is in turn controlled by element (f) and so on. Of course each of the control elements have their own control elements, such as (g) controlling the decay of control element (b). In turn, (g) is controlled negatively by a sequence of elements (h), (i), and (j) and positively by a positive control element (k). The elements illustrated here are only selectively presented for to include all of the infinite regression elements would not allow a clear picture of the infinite regression problem.

regression is present for both control of mRNA synthesis and mRNA degradation. A schematic illustration of this infinite regression problem is presented in Fig. 1.

PROBLEM OF VARIATION IN PROTEIN CONTENT DURING THE CELL CYCLE

Protein content variability during the cell cycle also illustrates a control-element problem. If mRNA for a particular protein were invariant during the cell cycle, a peak in protein content would require both a cell-cycle-dependent activator of translation before peak expression and a cell-cycle-specific protease after peak expression. Both of these control elements require further controls *ad infinitum*.

The breakdown of proteins after a peak in the cell cycle reveals an even more crucial problem. A specific protease acting after the peak of protein appearance requires a specific protease to destroy the initial protease; this allows the protein to increase during the next cell cycle. This proteolytic–antiproteolytic system would necessarily be cell-cycle dependent and protein specific.

RELATIONSHIP OF mRNA VARIATION TO PROTEIN VARIATION

If mRNA varied during the cell cycle, how would this mRNA variation affect protein changes during the cell cycle? The answer, oddly enough, is "not very much."

Equations describing protein variations, for both stable and unstable proteins (*22, 23*), during the cell cycle for different patterns of mRNA variation, demonstrate that even extremely large variations in mRNA produce only minimal protein variations during the cell cycle. The initial calculations concentrated on mRNA variations with a zero trough value (*22*). For large changes in mRNA content (*i.e.*, infinite amplitude with a sine wave trough or minimal value of zero) the maximal variation in protein content for a stable protein, compared to unregulated mRNA, is 22%. For a protein with a half-life of one-fifth the interdivision time, the maximal variation in protein content for large variations in mRNA is at most, threefold (*22*).

The original analysis (*22*) has been extended to patterns of mRNA variation with nonzero troughs (*23*). For these mRNA variations, the change in protein content is negligible. For example, for a trough value of 10 and amplitude of 2, the maximum deviation from unregulated mRNA for a stable protein is $\sim$2%. For an unstable protein (half-life equal to one-fifth of the interdivision time), the variation is $\sim$20% compared to an unregulated protein. Thus, a nonzero trough value for any sinusoidal variation strongly affects protein variation. When the minimal amount of mRNA is above zero, protein variation during the cell cycle essentially disappears (*23*).

The result of these calculations is that even if mRNA varied during the cell cycle, these changes cannot account for the observed changes in protein during the cell cycle. Because published data on mRNA variation during the division cycle generally do not give the absolute values of mRNA during the cell cycle, it is difficult to know precisely what one might expect for protein variation. The conclusion is that mRNA variation during the division cycle cannot produce significant variations in protein content during the cell cycle.

INVARIANT GENE EXPRESSION DURING THE CELL CYCLE—THE SOLUTION TO THE INFINITE REGRESSION PROBLEM

The solution to the problem of cell-cycle-variable gene expression (separate from cyclical protein content) is to postulate that gene expression (*i.e.*, mRNA content) is not cyclical but constant during the cell cycle. This proposal is at variance with the current, dominant, widely held consensus view of events during the mammalian cell cycle. The experimental evidence, however, and the theoretical considerations described here indicate that this proposal must be considered.

To re-examine the widely accepted view of cyclical gene expression, one must consider four points. First, the existence of cell-cycle variation in protein content does not mean that one must expect cyclical mRNA variation. That is, one cannot use the variation in protein content during the cell cycle to support the proposition that mRNA also varies during the cell cycle. Second, one must reconsider the data on mRNA variation during the cell cycle, with attention to problems of synchronization of cells and perturbations of cells when whole-culture methods are used (*24–38*). In particular, whole-culture synchronization methods cannot synchronize cells (*39*). Third, there are significant problems with using microarrays to measure mRNA during the cell cycle (*26, 22, 40–42*). And fourth, one must consider the logical and theoretical problems with postulating mRNA variation during the cell cycle as exemplified by both the infinite regression problem and the minor affect of mRNA variation on protein variation.

Much of the data on mRNA variation during the cell cycle is presented as "normalized" data, where the sinusoidal pattern is adjusted to a mean of zero and amplitude of 1.0. When this is done, the absolute values for the mRNA content during the division cycle are obscured. This means that one cannot predict the protein variation from any particular mRNA variation.

In addition to experimental problems, there has been a notable lack of consideration of the infinite regression problem that applies to the proposal that numerous genes have variable expression during the cell cycle. As described above, each proposed variation in mRNA expression requires the postulation of a cycle-specific variation in some control element. That control element in turn requires another cycle-specific control element, and so on. Until this "infinite regression" problem is considered and studied, it is difficult to understand how gene expression—that is, mRNA variation, not protein variation—can vary during the cell cycle. Overarching this general critique is the result that there are numerous problems with microarray assays; these have been described in detail (*40*).

PROBLEMS WITH mRNA ANALYSIS DURING THE CELL CYCLE

An example of problems with mRNA analyses can be seen in the work of Yang et al. (*43*). Their analysis of the results of Spellman et al. (*4*) indicated that the results are not reproducible and are very likely the result of perturbations of the cells by whole-culture synchronization methods. We have argued this case previously (*26*) but the visual evidence of Yang et al. (*43*), is revealing. In particular, the nonperturbing elutriation results suggest that the whole-culture methods have introduced cyclicities that do not exist in unperturbed cells.

mRNA CONTENT DURING THE UNPERTURBED CELL CYCLE

RT-PCR analysis of mRNA during the unperturbed cell cycle using cells produced by membrane-elution indicates that, in unperturbed cells, the mRNA content for seven cyclins is invariant during the cell cycle (*23*). Invariant gene expression during the cell cycle avoids the problem of having cycle-specific control elements postulated for mRNA variation that in turn require cycle-specific control elements.

Numerous measurements using microarrays have led to the proposal that myriad genes are expressed preferentially at different times or phases of the cell cycle. These proposed mRNA variations are insignificant in determining protein variation during the cell cycle. It is also important to consider a critique of the evidence for mRNA variation during the cell cycle. Much of this evidence is subject to the criticism that the synchronization methods used were perturbing and that the results are artifacts of the methods used.

One experiment that is likely beyond criticism, and cannot be dismissed are the results of Eward et al. (*44*) who used membrane elution and RT-PCR to conclude that the mRNAs of cyclins E, B1, and A2 vary cyclically during the cell cycle. The cells used in these experiments, a human cell line, MOLT-4, may be the reason for different results. A fundamental process of cell-cycle control and gene expression during the cell cycle would not be expected to vary between cells. One possibility for this result is the number of control genes used to correct for input RNA. Whereas Eward et al. (*44*) used only one control gene (18s rRNA), we have used four genes to determine the input RNA (*23*).

Our analysis of mRNA variation during the cell cycle using an automated, nonperturbing method for cell-cycle analysis indicates that there is no significant variation in gene expression during the cell cycle (*23*).

ANALYSIS OF PROTEIN VARIATION DURING THE NORMAL DIVISION CYCLE

The membrane-elution method was used to analyze proteins during the division cycle, specifically cyclins, and two significant observations were made (*16*). Cyclins A and B1 break down, or their antigenic specificity disappears (on Western blots), at the end of the cell cycle. Equally important, the significant breakdown at the end of the cell cycle is followed by the immediate resynthesis of these cyclins in the newborn cells and throughout the interphase of the cell cycle.

The immediate recovery of cyclin content in the newborn cells indicates that there is no proteolytic system that must be destroyed at the end of the cell cycle. Rather, it appears that there is something about the mitotic/cytokinetic period that allows breakdown, and when cytokinesis ends there is no further breakdown activity. This allows the immediate increase in protein content at the beginning of the cell cycle. By restricting protein breakdown to the window of mitosis/cytokinesis, one avoids the infinite regression problem.

The "mitotic window" model avoids the infinite regression problem because it does not postulate that a particular protease, such as the one that causes the disappearance of cyclin B1, must be destroyed after it performs its task during mitosis. Rather, the protease is proposed to exist throughout the cell cycle but works only at a particular time. One speculative model for such a mitotic window is that cyclin B1 binds only to condensed genetic material. In this bound form, the cyclin is susceptible to degradation by the existing protease. When mitosis ends, and the chromosomes decondense, the newly formed cyclin B1 is not degraded by the protease, as there are no condensed chromosomes in the cell. Thus, the infinite regression problem is avoided. The immediate reappearance of cyclin B1 in the newborn cells does not require the destruction of the specific protease that acts on cyclin B1.

A GENERAL MODEL OF PROTEIN AND mRNA VARIATION DURING THE MAMMALIAN CELL CYCLE

A succinct summary of the proposed cell-cycle model is that the increase in material during the cell cycle is a steady-state growth pattern. In this pattern of growth, all materials will increase in parallel and the ratio of any single molecule to any other molecule is constant. The only deviations observed from such a steady-state pattern are the protein breakdowns during a narrow window of the cell cycle. Other than this breakdown, the synthesis of all proteins and all mRNAs is invariant during the cell cycle.

Because protein is broken down only during a particular window of time—the mitotic phase—one avoids the infinite regression problems raised here. The control enzymes may always be present and need not be removed; they would work only during the mitotic window. This avoids the need to postulate any control element activating or destroying the protease that breaks down particular proteins (*16*).

The steady-state model eliminates the infinite regression problem, or paradox, as there is no need to postulate any cycle-dependent controls that would in turn require cell cycle dependent controls. For the vast majority of material in the cell cycle, specifically the cytoplasmic components, it is proposed that the rate of increase in each component (excluding the genome) is invariant during the cell cycle. As mass increases exponentially (*45, 46*), with some few exceptions such as cyclins A and B1 (*16*), the cell components all increase steadily and in parallel. Newborn cells are presumed to have a unit amount of each cell component and twice as much at the instant of division. The newborn cells produced by division have a unit amount of each material. The doubling of cell material between birth and division is *a priori* obvious, with the only question being the pattern of material increase during the division cycle. Figure 2 illustrates the main points of this model.

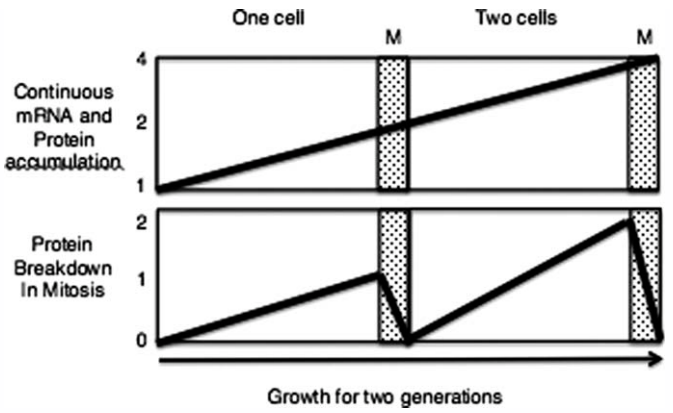

Figure 2. Illustration of continuous mRNA and protein accumulation with particular proteins breaking down during mitosis. The upper figure shows continuous exponential accumulation over two generations. The lower figure shows breakdown of some proteins during the mitotic period (M, shaded area).

RELATIONSHIP OF THESE IDEAS TO OTHER WORK ON THE CELL CYCLE

I recognize that the findings and proposals presented here are different from the widely accepted findings that some or many mRNAs are formed periodically in the eukaryotic cell cycle. These widely accepted findings have been made in many laboratories using many different techniques for cell-cycle analyses and mRNA measurements. By comparison, the results described here are quite limited. Even published results on mRNA variations should be considered subject to reexamination. One of the best examples of the problems with mRNA measurements comes from a reanalysis of work with human cells (*5*). It was shown (*42*) that the mRNA variations were the result of random experimental variations and the cells were not truly synchronized. A more complete analysis of the general use of microarrays to analyze the cell cycle has been published (*40*). Most important are recent results using PCR analysis of unperturbed synchronized cells to demonstrate (with a small set of genes) that mRNA content is invariant during the cell cycle (*23*).

AN ALTERNATIVE VIEW OF THE CELL CYCLE

The ideas presented here are at variance with the current, dominant, consensus view of the cell cycle. The current view is that there are numerous checkpoints, restriction points, variations

in protein and mRNA content during the cell cycle, and other cell-cycle events. In previous publications, we have dealt with such elements as the restriction point and the G0 phase (*47*), as well as cyclical phosphorylation of Rb protein (*48, 49*). Equally important are critiques of the most commonly used whole culture methods for synchronization (*28, 29, 32, 34, 35, 39*). The sum of these critiques have led to an alternative view of the cell cycle that does not include many of the widely accepted cell-cycle control systems (*24*).

TRIGGERING OF CELL-CYCLE EVENTS DURING STEADY-STATE GROWTH

The proposal of steady-state, continuous, and uneventful growth during the mammalian division cycle raises the question: "How are events such as initiation of S phase or initiation of mitosis triggered?" If one eliminates the cycle-specific increase in some cellular element, how do events get initiated? Although a criticism of one model does not require the production of an alternative or substitute model, it may be helpful to consider a simple alternative that will support the critique described above.

The model proposed here is that initiation of events during steady-state passage is related to the continuous accumulation of some triggering element in the cell, not the phase- or time-dependent appearance of some triggering element. Whatever the ultimate initiator of DNA synthesis, and whatever the ultimate initiator of mitosis, it is the steady-state accumulation of some material that leads to the initiation of S phase and the eventual initiation of mitosis. In this view, cell-cycle events are triggered by a quantitative change in the triggering element rather than its appearance at a particular time during the division cycle. It is possible that the completion of S phase is the ultimate trigger of mitosis, in which case only the initiation of S phase itself has to be accounted for.

METAPHORS OF THE CELL CYCLE

One metaphor of the analysis presented here is the Russian Doll model (*23*). The widely accepted gene control system is like nesting Russian Dolls. These dolls are called *matryoshka* in Russian. The outer doll is generally some grandmotherly figure that when opened reveals another smaller doll of another figure and when that is opened another doll appears. The nesting dolls are a visual metaphor for the currently postulated sequence of control elements required to produce a cyclical or periodic pattern of gene expression. Just as opening one doll reveals another doll, so postulating one solution to the cycle-specific variation leads to another problem, the cycle-specific appearance of additional control elements. Similarly, further problems are revealed, just as one finds more and more dolls nested in the Russian Doll set (Fig. 3a).

The apocryphal story of "turtles all the way down," recently popularized by Stephen Hawking offers another example. A well-known scientist (some say it was Bertrand Russell, others say William James) once gave a public lecture on astronomy. He described how the earth orbits around the sun and how the sun, in turn, orbits around the center of a vast collection of stars called our galaxy. At the end of the lecture, an audience member at the back of the room got up and said: "What you have told us is rubbish. The world is really a flat plate supported on the back of a giant tortoise." The scientist gave a smile before replying, "What is the tortoise standing on?" "You're very clever, young man, very clever, but it's turtles all the way down!" (Fig. 3b).

Figure 3. Illustration of metaphors of infinite regression. (a) Nesting Russian dolls (b) "Turtles all the way down."

EXPERIMENTAL FACTS AND THEORETICAL ARGUMENTS

It is important to deal with what may be the central concern of readers. This has been expressed clearly by an anonymous reviewer who wrote: "**. . .**irrespective of possible problems of synchronization or perturbation, constant gene expression as postulated must show in the measurements, and it does not, irrespective of the method used for detection." As I see it, this argument stems from the belief that published work based on experimental methods is indisputably correct. However, the analysis presented here should be considered despite what is generally regarded as indisputable evidence for cell-cycle variations in gene expression. Negative results are sometimes not published. There may be a bias toward publishing experimental results that show cell-cycle variations. These results are easier to write about and explain within the current or "standard" model of the cell cycle.

FUNCTION OF HEURISTIC PROPOSALS

It is not proven in this analysis that there are no or few genes that are expressed in a cell-cycle manner. Such patterns may exist, and it is not possible to prove a universal negative by experimental means alone. Nevertheless, I write here from

skepticism regarding the existence of a number of cyclically expressed genes. The proposal of cell-cycle-dependent patterns of gene expression must ultimately grapple with the problems raised here. This analysis places the burden of proof regarding the existence of cyclically expressed genes on those who propose that these patterns do exist.

What is the meaning and source of the word "heuristic" as used in the title of this article? Heuristic refers to a hypothesis that serves as a guide and gives direction to solving a problem but is not considered proven (*50*). No theoretical argument can cancel out experimental results. But such results, unlike mathematical proofs, may be overturned by additional experimental work. The purpose of this article is not to prove that such patterns do not exist, but to raise the questions that bring into focus key problems regarding the control of the cell cycle that have not been generally considered.

If there are arguments against the questions raised here, I look forward to an explicit analysis that will either answer the objections or problems raised here or show experimentally that the postulated control elements do, in fact, exist.

This analysis is a critique of the current belief system regarding the cell cycle. Therefore, it is appropriate to consider how belief systems affect critiques of that system. John Kenneth Galbraith, the economist, has written a beautiful description of this process:

"The emancipation of belief is the most formidable task of reform and the one on which all else depends. It is formidable because power that is based on belief is uniquely authoritarian; when fully effective, it excludes by its nature the thoughts that would weaken its grasp. It can also be pleasant—a womb in which the individual rests without pain of mental activity or decision. Or, to change the metaphor, as with Tolstoy's happy soldier, all personal responsibility is given over to the regiment. And the drums to which all march are those of others**...**"

A particularly apt example of this process is seen in a recent article (*13*). These authors cited a article on the cyclical expression of genes (*5*) as support of the current model. They also cited another article (*42*) that was described as proposing "Early efforts to analyze human cell cycles had mixed success**...**" The second article, however, was actually a demonstration that the experimental work on human cell cycles (*5*) was invalid because the results were not reproducible, and the cells were not actually synchronized (*42*). Another example of misunderstanding comes from a article (*51*) which attributed to the article from this laboratory (*42*) the proposal that "a large number of genes would be regulated in a cell-cycle-specific manner in normal and cancer cells." Our laboratory article (*42*) proposed exactly the opposite. In addition, their article (*51*) used a double thymidine block to synchronize cells when previously published articles have shown that such an approach is both theoretically (*39*) and experimentally (*34*) unable to synchronize cells.

These examples, as well as others not cited here, show the difficulty that ideas not commonly discussed in the literature have in being understood and cited properly. The analysis presented here seeks to restore an alternative viewpoint to a proper place of scientific discussion in the cell-cycle community.

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