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Cell Biology International 33 (2009) 10–18

Cell Biology International

www.elsevier.com/locate/cellbi

Invariant mRNA and mitotic protein breakdown solves the Russian Doll problem of the cell cycle

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Received 18 June 2008; accepted 13 October 2008

Abstract

It has been proposed that cyclical gene expression occurs at a large number of different times during the cell cycle. The existence of a large number of cycle-specific variations in mRNA and protein during the eukaryotic cell cycle raises the problem of how cell-cycle variations are regulated. This is the ''infinite regression'' or Russian Doll problem where postulating a cell-cycle specific control element pushes the explanation of cell-cycle variation back one step to the problem of how that control element varies during the cell cycle.

PCR studies on unperturbed cells indicate Cyclin mRNA content is invariant during the cell cycle. Furthermore, calculations reveal that variations in mRNA content do not account for observed protein variations.

Continuous and constant gene expression during the cell cycle, continuous protein accumulation, and protein breakdown only within the mitotic window solves the Russian Doll problem or infinite regression problem. These results, and theoretical ideas support an alternative view of the cell cycle where many of the proposed control systems do not exist.

Keywords: Cell cycle; Eukaryotic cells; Gene expression; Regulation

1. Introduction

It is widely accepted that a large number of genes are expressed at different times during the cell cycle. We raise a key question regarding the control of gene expression at different times during the cell cycle. This is the Russian Doll problem presented in detail below.

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* (Cho et al., 1998; Spellman et al., 1998), different groups have studied such diverse eukaryotic cells as primary human fibroblasts (Cho et al., 2001), HeLa cells (van der Meijden et al., 2002; Whitfield et al., 2002), *Arabidopsis thaliana* (Breyne et al., 2002), and *S. pombe* (Marguerat et al., 2006; Oliva et al., 2005; Peng et al., 2005; Rustici et al., 2004), as well as the prokaryote *Caulobacter crescentus* (Laub et al., 2000). 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.

And even more to the point, these numerous patterns of proposed cyclical gene expression occur in a continuous manner (Holter et al., 2000; Spellman et al., 1998) so that there must be controls regulating the timing of gene expression at numerous points throughout the cell cycle. If cyclical gene expression occurred in a small number of groups, one could imagine a small number of controls for the groups of cyclically expressed genes. But the problem becomes more difficult when a large number of genes are expressed at many different times during the cell cycle.

In addition to mRNA variations, there are also variations in protein content during the cell cycle. Proteins have been

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doi:10.1016/j.cellbi.2008.10.004

classified by the time or phase during the cell cycle at which protein content peaks or is rapidly synthesized (Darzynkiewicz et al., 1996; Ohtsubo et al., 1995; Sherwood et al., 1994; Takita et al., 2003). 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 protein breakdown during the cell cycle has also pointed out that many proteins break down specifically during mitosis (Pines, 2006).

The question thus arises as to how myriad cyclical gene expression patterns and protein variations (which presumably regulate passage through the cell cycle) are themselves regulated during the cell cycle. Consider a gene whose expression (i.e., mRNA content) varies during the cell cycle, with expression maximal at some particular cell-cycle age or phase. In order for this change in rate of mRNA synthesis from a specific gene to occur there must be the appearance of some cellular element—let us call this ''control element #1''—that controls that gene's expression or content and which changes the rate of mRNA synthesis from this gene. At some later time this element must cease its activity in order to cease mRNA synthesis and allow the extant mRNAs to decay. This scenario would give a cyclical pattern of gene expression for a particular gene. If the change in mRNA content were due to a change in the decay kinetics of mRNA, a change in the rate of breakdown (i.e., a decrease in the rate of breakdown) would lead to an increase in the content of a specific mRNA. This breakdown change would also require the cycle-specific appearance of a cellular control function related to the breakdown of a specific mRNA.

How is control element #1 itself regulated? To explain mRNA variation one must postulate some increase in control element #1 (assuming it is a positive control element) to stimulate mRNA synthesis. Later in the cell cycle this control element #1 must disappear or be inactivated so that mRNA synthesis ceases or decreases. Control element #1 is presumably regulated by ''control element #2''. But control element #2 itself then requires a cycle-specific control system, which we consider control element #3. Continuing this process we could imagine control elements #4, #5, and so on, ad infinitum.

This reasoning can also be applied to changes in the breakdown of mRNA, where cycle-specific changes in control elements require prior cycle-specific changes, ad infinitum. Since the genes that are cyclically expressed generally have different times of peak expression, one can postulate separate sets of control elements 1, 2, 3, … *n*, for each proposed gene that varies during the cell cycle.

Variation in protein content during the cell cycle provides a similar problem regarding control elements. Thus, if mRNA were invariant, a peak in protein content at a particular time during the cell cycle would require the cycle-dependent appearance of some activator of protein translation from the extant mRNA and the appearance of a specific protease after peak expression. Both of these control elements would require further controls and so on, ad infinitum.

The breakdown of proteins after a peak during the cell cycle reveals an even more crucial problem. The postulation of a specific breakdown activity or protease acting after the peak of protein appearance would require some specific anti-protease or a specific protease that breaks down the first protease in order to allow the protein to increase during the next cell cycle. This proteolytic-anti-proteolytic system would be cell-cycle dependent and again we have the problem of further controls acting during the cell cycle.

These problems, as a group, are examples of the ''infinite regression'' problem where the proposed solution to one problem leads to further problems. A physical metaphor for this problem can be seen in the popular Russian Dolls. The gene control system described here is like the nesting Russian Dolls that are now ubiquitous in the world. They are sometimes called nested dolls or stacking dolls or matryoshka in Russian; it is probably the most popular Russian national souvenir. The outer doll is generally some grandmotherly figure that when opened reveals another smaller doll of another figure and when this is opened another doll appears. The nesting dolls are a visual metaphor for the postulated sequence of control elements required to produce a cyclical or periodic pattern of gene expression. So postulating one solution to the cycle-specific variation leads to another problem, the cycle-specific appearance of the control elements for the first problem, and then another, and so on.

2. Materials and methods

2.1. Cells

L1210 cells, a mouse leukemic line (ATCC designation CCL219) was used for all experiments. The cells are non-adherent and grow with a doubling time of 9–11 h.

2.2. Media

Liebovitz's L-15 medium (cellgro by Mediatech, Herndon, VA 20171) was supplemented with 2 mg/ml glucose, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% Cosmic Calf Serum (CCS, (Hyclone, Inc.)). The buffering in L-15 medium allows cell growth and pH maintenance without a CO_2 atmosphere. Cells were grown at 37 °C in sealed flasks prior to a membrane-elution experiment. Cells were kept <200,000 cells/ml during exponential growth. The CCS was filtered (0.22 or 0.45 μm pore filters) before a membrane-elution experiment to avoid clogging the membrane.

2.3. Membrane holder

The membrane holder apparatus has been described previously (Eward et al., 2004; Helmstetter et al., 2003; Thornton et al., 2002), but some of the details will be presented here. A support screen (Millipore, catalogue number YY3014234) was secured in a holder with rubber gaskets so that a membrane (Millipore catalogue number GSWP14250; 142 mm nitrocellulose membrane, 0.22 μm pores) lay directly on the support screen. A Lucite ring confined the liquid to the top of the membrane. Rubber gaskets between the membrane

and Lucite ring prevented leakage. The support screen lay over a funnel that can be inserted into a side-arm flask to allow suction to pull the medium through the membrane (Fig. 1).

2.4. Automated membrane-elution

Cells were grown to a concentration of <200,000 cells per ml to obtain 60–70 million cells. For example, 600 ml of cells at 100,000 cells per ml gave 60 million cells. All experiments were carried out in a 37 °C room, with warm media and buffers. The membrane holder and medium reservoir were kept in a full-view incubator within the warm room to ensure constant temperature. To start the production of newborn cells, 50 ml of PBS (phosphate-buffered saline) with 10 μg/ml concanavalin A was filtered through the membrane. Upon completion of the filtration no residual liquid remained. PBS (100 ml) was then filtered through the membrane to remove unbound concanavalin A; again no residual liquid remained. Cells in 300–600 ml of medium were filtered slowly onto the membrane with gentle suction over 3–5 min. When 20–30 ml of liquid remained above the membrane, the liquid was poured off so that the cells were never dried and exposed to air. The membrane apparatus was inverted and filled with fresh medium. Medium from a 4 L reservoir was pumped through the membrane at 2.5–3.0 ml/min. After ~30 min, the unbound and weakly bound cells had been removed. The unbound cells obtained from this initial flow of medium through the membrane were collectively referred to as the "wash-off". The wash-off was usually 10–20% of the input cells. Thus, >80% of the initial cells were bound to the membrane. The eluted cells were monitored until it was determined that only newborn cells were eluted as determined by cell size. Then the membrane was placed over a large funnel connected by tubing to a peristaltic pump. The pump connected to the bottom of the funnel pumped liquid at 4.0–10.0 ml/min to prevent pooling of cells in the funnel. The eluate from the membrane was collected in sterile glass vials (40 ml) in a Pharmacia fraction collector. Although the entire system (medium reservoir, pumps, membrane holder, fraction collector) was in a warm room, an incubator box was built around the fraction collector. The incubator box contained a thermocouple-controlled heater with a fan to maintain a constant temperature. Thus, even when the warm room door was occasionally opened, there was no change in the temperature of the collected cells.

Fractions were collected for 15 min intervals yielding 35–40 ml of media with newborn cells. The concentrations of cells in each vial were generally <25,000/ml; no inhibition of growth occurs at this low cell concentration. Since cells in each of the vials in the fraction collector grew for different lengths of time prior to cell harvesting, each vial contained cells at different cell-cycle ages (Fig. 1). At the end of a collection period (15–18 h), the vials were placed in an ice bath. The cells were collected by centrifugation for analysis of cell sizes and mRNA content by RT-PCR. Previous studies on protein content referred to in the discussion were analyzed in the same way, with cells collected from the automated membrane-elution apparatus at various cell-cycle ages.

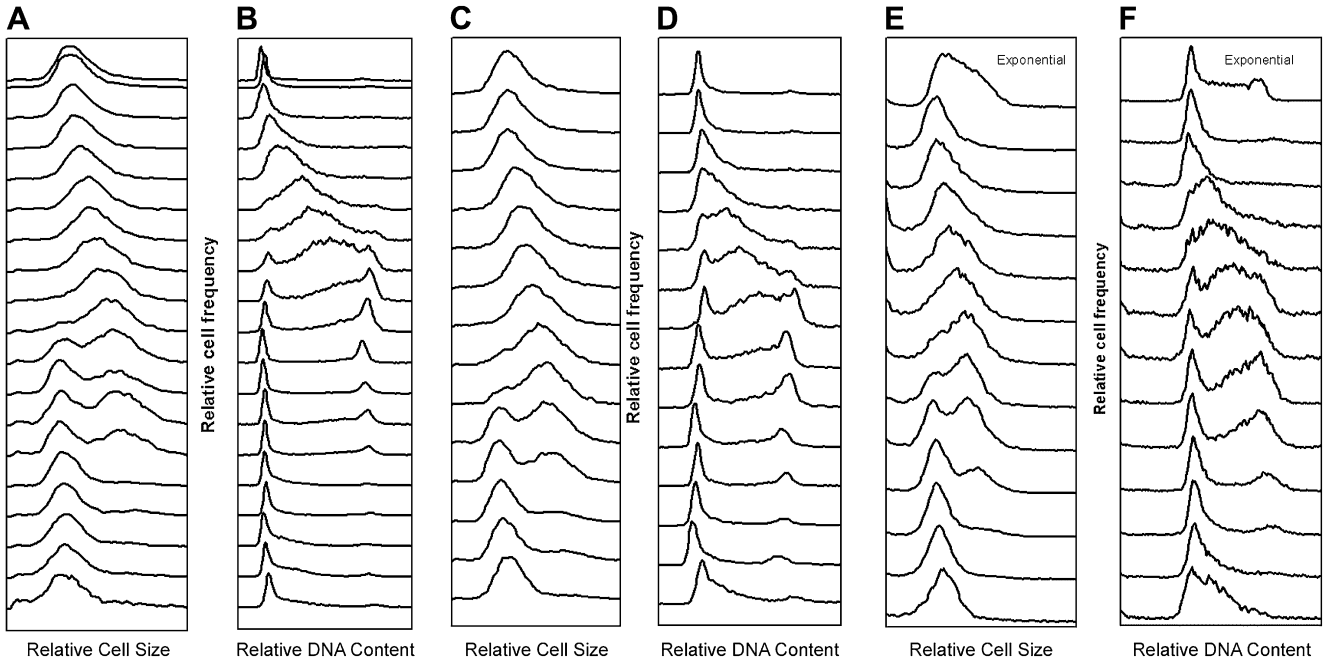

Fig. 1. Changes in the size distribution and DNA content of cells during the cell cycle. Three independent experiments are presented (A–B, C–D, E–F). Each line is separated in time by one hour in graphs A–D, and by 1.33 hours in graphs E–F. (In graphs A and B the first two lines are separated by fifteen minutes with subsequent lines separated by one hour.) Newborn cells at the top are from the last fraction collected from the automated membrane-elution system. Each subsequent line represents cells that have grown for one hour longer than the previous line (or 1.33 hours in graphs E and F.) The size distributions in panels A, C, and E correspond with the respective DNA distributions in panels B, D, and F. Exponential size and DNA distributions are shown at the top of panels E and F. The results shown in A–D are for 10% CCS while the panels E and F used 5% CCS. (reprinted from (Cooper et al., 2007)).

2.5. Cell counting and sizing

Cells were counted and sized in L-15 medium using a Beckman/Coulter Z2 Particle Counter and Size Analyzer. The data were collected and analyzed using the Z2 AccuComp program from Beckman Coulter (version 3.01). Size distributions were exported from the AccuComp data to an Excel spreadsheet program for analysis and plotting.

2.6. mRNA assay by RT-PCR

Total cellular RNA was extracted with the RNeasy mini Kit (Qiagen) following the manufacturer's protocol. To avoid amplification of residual genomic DNA, the mRNA extract was treated Ambion's TURBO DNA*free* ™ DNase Treatment according to the manufacturer's protocol. First strand cDNA was generated using Reaction Ready First Strand cDNA Synthesis Kit (Superarray Inc.) with 1 ug of total RNA. Real time PCR was performed on an ABI 7900HT using the Mouse Cell Cycle PCR array (Mouse PCR Mouse PCR Array APMM-02 from the Superarray Bioscience Corporation) according to the PCR Array User manual. Baseline, threshold, and CT values were calculated automatically by the SDS 2.2.1 software; the data were normalized using an average of 4 control genes.

3. Results

3.1. Experimental analysis of mRNA variation during the division cycle for specific cyclins

We have measured the mRNA content for many genes believed to be associated with the cell cycle using the Superarray system. The mRNA analysis was determined on cells produced without perturbations using an automated membrane-elution (''baby-machine'') system. Patterns of cell size change and DNA change during the cell cycle from these cells are shown in Fig. 1. These size and DNA patterns indicate that the automated membrane-elution method produces cells that are newborn and that grow as expected through the cell cycle. No starvation or inhibition or other perturbations are used on these cells.

The Superarray system of mRNA assay by RT-PCR yields information on 84 genes for the mouse cell-cycle system. There are 12 control genes used to correct minor changes in mRNA input. We present results for a limited subset of those genes, those of the cyclins. In Fig. 2, the normalized mRNA contents during the cell cycle indicate that there is no observed fluctuation of mRNA content during the cell cycle. The results in Fig. 2 indicate that the mRNA concentrations for the cyclins listed are constant during the cell cycle and therefore the mRNA content increases exponentially during the cell cycle. This is because the cell volume and mass increase exponentially during the cell cycle (Cooper, 2006)

Most important for this analysis is the observation that for any variation there is no zero trough value. This means that the variation in protein during the division cycle from these mRNA molecules is expected to be negligible (see Section 3.2, and Section 4 below on presentation of mRNA data during the cell cycle).

3.2. Analysis of relationship of mRNA variation during division cycle to protein variation

If mRNA did vary during the cell cycle, how would this mRNA variation affect protein variation during the cell cycle? The answer is ''not very much''.

We have derived equations for the variation of proteins during the cell cycle for particular patterns of mRNA variation during the cell cycle, and for both stable and unstable proteins (Cooper and Shedden, 2007). Those initial calculations primarily were concentrated on mRNA variations with a zero trough value. For extremely large changes in mRNA content (i.e. infinite amplitude, with a trough in the sine wave pattern

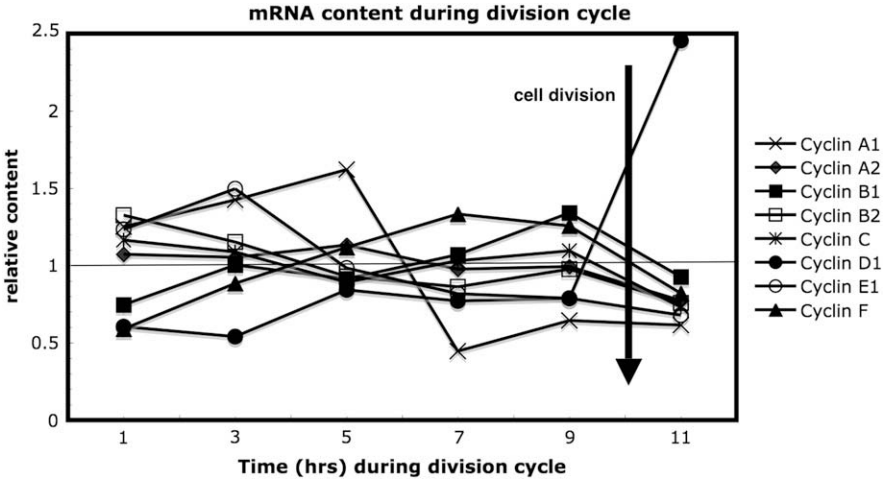

Fig. 2. mRNA content during the cell cycle. RT-PCR analysis of mRNA contents were assayed using the superarray system as described in the methods. The calculated contents of mRNA were averaged and the values were divided by the average to put all the values around a value of 1.0. The relative changes in the mRNA contents are indicated in the graph, although the absolute values for each mRNA are quite different.

of zero value), the maximal variation in protein content for a stable protein compared to unregulated mRNA is at most 22%. For protein that has a half-life of one-fifth the interdivision time, the maximal variation in protein content for extremely large variations in mRNA is at most 3-fold.

We then extend the original analysis to patterns of mRNA variation that include patterns with non-zero trough. For a set of mRNA patterns, the expected protein variation during the cell cycle is calculated. The variation in protein content during the cell cycle for both stable and unstable proteins is shown in Fig. 3. For mRNA variations with a non-zero trough value the change in protein, compared to an unregulated protein, is quite minimal. For example, with a trough value of 10 and amplitude of 2, the maximum deviation from unregulated protein for a stable protein is ∼2%. For an extremely unstable protein (half-life equal to one-fifth of the interdivision time) the variation is ∼20% compared to an unregulated protein.

What is most important to recognize is how a non-zero trough value for any sinusoidal variation affects the expected protein variation. When the minimal amount of mRNA is above zero the change in protein during the cell cycle essentially disappears.

The conclusion from these calculations is that even if mRNA did vary during the cell cycle, these changes cannot account for the larger observed changes in the protein content 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 of this quantitative analysis is that all of the work of mRNA variation during the division cycle does not seem to be able to produce variations in protein.

4. Discussion

4.1. Invariant gene expression during the cell cycle—the solution to the infinite regression or Russian Doll problem

The solution to the problem of cyclical gene expression (separate from cyclical protein content) is to postulate that gene expression (i.e. mRNA content or production) is not cyclical, but is constant during the cell cycle. This proposal is at variance with the prevailing dominant (consensus) view of events during the mammalian cell cycle, but the evidence and the theoretical considerations raised here suggest that our proposal must be carefully considered.

In order to reconsider the widely accepted view of cyclical gene expression, let us consider three points. First, the clear presence of cell-cycle variation in protein content does not mean that cyclical mRNA variation is expected. Second, the data on mRNA variation during the cell cycle has to be reconsidered, with attention to problems of synchronization of cells and perturbations when whole-culture methods are used (Cooper, 1998c, 2002, 2003a,b, 2004c,d, 2005, 2006), as well as problems with microarrays (Cooper and Shedden, 2003;

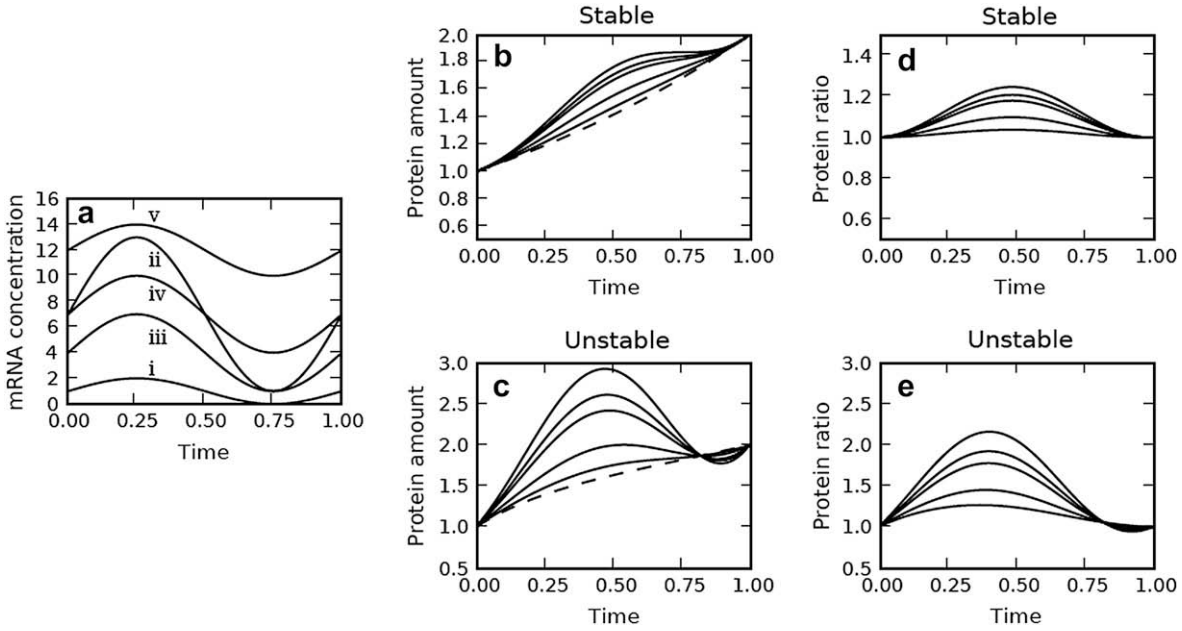

Fig. 3. Relationship of mRNA to protein during the cell cycle. Panel A is a selection of five sine wave patterns that have different amplitudes and minima. They are labeled from the sine wave pattern that gives the largest variation in protein (i.e., (i), to that with the least variation (i.e. (v)). The values (c = minimum and A = amplitude, where amplitude is one half the peak to trough value) for the sine waves are: (i) $c = 0$, $A = 1$, (ii) $c = 1$, $A = 6$ (iii) $c = 1$, $A = 3$, (iv) $c = 4$, $A = 3$, (v) $c = 10$, $A = 2$. All of these sine waves, when normalized to a mean of zero and an amplitude of 1.0 give the same pattern; they differ in the absolute values of the troughs and peaks. All sine waves with a trough of 0.0 give the same protein pattern. Panels B and C show the calculated protein amount per cell during the cell cycle for protein made in proportion to the extant mRNA in Panel A. Panel B are the results for stable proteins, and Panel C are the results for unstable proteins with a half-life equivalent to one-fifth the interdivision time. The dashed lines in Panels B and C are the amount of mRNA for a gene whose expression is unregulated and which gives an exponential increase in mRNA during the cell cycle. Panel D (stable proteins from Panel B) and Panel E (unstable proteins from panel C) are the calculated ratios of protein relative to an unregulated protein (dashed lines in Panels B and C) made exponentially during the cell cycle.

Shedden and Cooper, 2002a,b). And finally, 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.

The data on variation in mRNA content during the cell cycle has generally been based on whole-culture methods of synchronization and the use of microarrays to analyze mRNA from numerous genes. Without reviewing the argument in its entirety, it should be pointed out that whole-culture methods cannot synchronize cells (Cooper, 2003a). Experiments testing some popular methods for synchronization have supported the conclusion that whole-culture methods cannot synchronize cells (Cooper, 2002; Cooper et al., 2008, 2006; Liliensiek et al., 2006). The lack of synchronization is a problem in addition to the probable introduction of perturbations when whole-culture methods (usually starvation or inhibition) are used to analyze the cell cycle. The use of microarrays has also been questioned, with the data on mRNA variation during the cell cycle being strongly criticized (Cooper and Shedden, 2003; Shedden and Cooper, 2002a,b).

It also needs to be understood that much of the data on mRNA variation during the cell cycle has presented ''normalized'' data, where the sinusoidal pattern are adjusted to a mean of zero and an amplitude of 1.0. When this is done, the absolute values for the mRNA content during the division cycle are hidden, i.e. one cannot predict the protein variation from that particular mRNA variation.

In addition to these experimental problems, there has been a notable lack of consideration of the infinite regression problem that adheres to the proposal that numerous (hundreds to thousands) of genes have variable expression during the cell cycle. Each proposed variation in mRNA expression requires the postulation of a cycle-specific variation in some control element, and that control element in turn requires another cycle-specific control element, and so on. Until this ''infinite regression'' problem exemplified by the Russian Doll metaphor is considered and studied, it is difficult to understand how gene expression—again, mRNA variation, not protein variation—can vary during the cell cycle. A more general critique of mRNA analyses during the cell cycle rests on two points. First, most of the data is presented as normalized data so that the absolute values for mRNA content during the division cycle are not readily available. Thus one does not know whether or not a trough has a zero value for mRNA content or a very large, non-zero value. Second, there are numerous problems with microarray assays, and these have been described in detail elsewhere (Cooper and Shedden, 2003).

4.2. 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. (2005) where an analysis of the results of Spellman et al. (1998) 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 previously argued this case (Shedden and Cooper, 2002a), but the visual evidence of Yang et al. (2005) is revealing. In particular, the non-perturbing elutriation results suggest that the whole-culture methods have introduced cyclical activities that do not exist in unperturbed cells.

4.3. mRNA content during the unperturbed cell cycle

RT-PCR analysis (Fig. 2) indicates that in unperturbed cells the mRNA content for seven cyclins is invariant during the cell cycle. By not having gene expression vary during the cell cycle we avoid the problem of having cycle-specific control elements postulated for mRNA variation that in turn would require cycle-specific control elements.

The numerous measurements using microarrays have led to the proposal that hundreds and possibly thousands of genes are expressed preferentially at different times or phases of the cell cycle. We have noted above that such mRNA variation is very likely insignificant with regard to protein variation during the cell cycle. But aside from that, we have to consider the evidence for mRNA variation during the cell cycle. Much of this evidence is subject to the criticism that the methods used were perturbing and that the results are artifacts of the methods used.

One experiment that is probably free of the above criticism, and thus cannot be lightly dismissed comes from Eward et al. (2004), who used membrane elution (the method used here) and RT-PCR (also used here) 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, could be accounted for as the reason for different results. We do not feel that such a fundamental process of cell-cycle control and gene expression during the cell cycle would vary between cells, even when the cell lines are mouse and human cells. One difference is the number of control genes used to correct for input RNA. Whereas Eward et al. (2004) used only one control gene (18s rRNA), we have used 4 genes to determine the input RNA. While one may quibble with the use of 18s RNA as a normalization control, the fact that cyclin E varies differently from cyclins B1 and A indicates that this is not apparently the problem. At this time we cannot explain the difference between our results and that of Eward's group except to say that the theoretical considerations of the infinite regression problem support the experimental results described here.

4.4. Analysis of protein variation during the normal division cycle

We have used the membrane-elution method to analyze various proteins during the division cycle, specifically cyclins, and have made two significant observations (Cooper et al., 2007). First, 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 essentially 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 suggests that there is no proteolytic system that has to 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 now allows the increase in protein during the beginning of the cell cycle. By restricting protein breakdown to mitosis and cytokinesis, one of the central infinite regression problems is avoided, the cycle-specific removal of a protease in order to allow protein synthesis to proceed in subsequent cycles.

4.5. A general model of protein and mRNA variation during the mammalian cell cycle

A succinct summary of the model of the cell cycle that we propose is that the increase in material during the cell cycle is essentially a steady-state growth pattern. In a steady-state pattern of growth during the cell cycle all materials will increase in parallel and the ratio of any single molecule to any other molecule would be constant. The only deviations we would observe from such a steady-state pattern are the breakdown of proteins during a narrow window of the cell cycle (Fig. 4). Other than this breakdown, the synthesis of all proteins and all mRNAs is invariant during the cell cycle.

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, we propose that the rate of increase in each component (excluding the genome) is invariant during the cell cycle. As mass increases exponentially (Cooper, 1998b, 2004b), it is proposed (with some few exceptions, such as cyclins A and B1), that 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. Given these constraints, the newborn cells produced by division have a unit amount of each material. The doubling of cell material between birth and division is presumably *a priori* obvious, with the question only being the pattern of material increase during the division cycle.

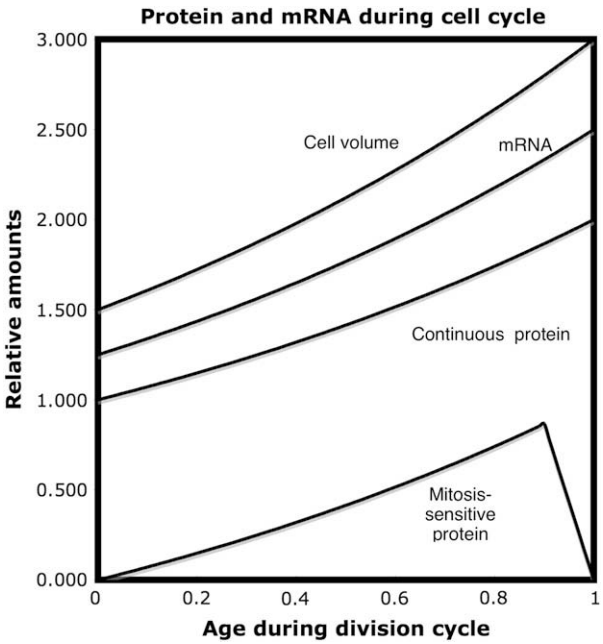

Fig. 4. Summary of proposed protein and mRNA variations during the cell cycle. Cell volume increases exponentially. For those mRNAs and proteins that are not cell-cycle regulated they increase exponentially as well, so that the concentration of both the proteins and mRNAs remain invariant during the cell cycle. Those proteins that are cell-cycle variable break down at the end of the cell cycle during the mitotic/cytokinetic window. For all four lines in this graph, the value at the end of the cell cycle is twice that of the values in the newborn cells, so that at division the newborn cells start the cycle with the proper amount of protein.

The predominant view today of the cell cycle is that numerous genes are expressed at particular times during the cell cycle. The sequential activation of different genes at different times is believed to lead to the eventual cell division. In contrast, we propose that there is no cell-cycle variation in gene expression. All genes are expressed at a constant rate at all times during the cell cycle. Exceptions from the absolute total steady-state pattern are the breakdown or loss of some proteins during mitosis. These proteins are synthesized in a constant manner, as the other proteins are, but because of breakdown at mitosis, the ratio of these mitotically-sensitive proteins to other proteins is not constant, as would be required in true steady-state conditions. The important point is that even the proteins that appear to be lost at mitosis are not made at any particular time during the cell cycle but are made continuously in proportion to the extant mRNA. The only difference is that, rather than increasing from unit amount to 2 units at division, they increase from essentially zero amount at birth to a maximum just prior to mitosis, during which they are destroyed. A summary of the proposed pattern of mRNA and protein increase during the cell cycle is presented in Fig. 4.

4.6. Relationship of these experiments to other work on the cell cycle

We 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 different findings have been made in many laboratories using many different techniques for cell cycle analyses and mRNA measurements. By comparison, the results here are quite limited. But even published results on mRNA variations should be considered to be subject to reexamination. One of the best examples of the problems with some mRNA measurements comes from a reanalysis of work with human cells (Cho et al., 2001) where it was shown (Shedden and Cooper, 2002b) that the mRNA variations were the result of random experimental variations and that the cells were not truly synchronized. A more complete analysis of the general use of microarrays to analyze the cell cycle has been published (Cooper and Shedden, 2003). While the experimental results here are numerically much less than

experiments that have hundreds of genes analyzed, we suggest that the Russian Doll problem is still present and needs to be considered.

4.7. Alternative views of the cell cycle

We explicitly point out that the ideas presented here are at variance with the present consensus view of the cell cycle where there are numerous checkpoints, restriction points, proposed variations in protein content during the cell cycle, proposed variations in mRNA content during the cell cycle, cyclical activation of various proteins during the cell cycle and other proposed cell-cycle events. We have dealt with such elements as the restriction point and the G0 phase (Cooper, 1998a), and cyclical phosphorylation of Rb protein (Cooper and Shayman, 2001; Cooper et al., 1999). But even more important are critiques of the most commonly used method for synchronization, the whole-culture methods (Cooper, 2002, 2003a, 2004a,c, Cooper et al., 2008, 2006). The sum of these critiques lead to an alternative view that does not include many of the widely accepted cell-cycle control systems (Cooper, 2000). Therefore it is not a critique of the ideas proposed here that they do not conform to the current view of the cell cycle; rather the ideas presented here offer an alternative view of the cell cycle and its regulation.

4.8. Triggering of cell-cycle events during steady-state passage

The question that the proposal of steady-state growth during the mammalian division cycle raises is: ‘‘how are events such as initiation of S phase or initiation of mitosis triggered?’’ The answer proposed here is that initiation of events during steady-state passage is related to the continuous accumulation of some triggering element in the cell, rather than the phase- or narrow-time-dependent appearance of some triggering element. Whatever the ultimate initiator of DNA synthesis, and whatever the ultimate initiator of mitosis, we propose that it is the steady-state accumulation of this material that leads to the initiation of S phase and the eventual initiation of mitosis. This proposes that it is a quantitative measure of the triggering element, rather than a qualitative change in the triggering element with its appearance occurring at a particular time during the division cycle. It is possible that the completion of S phase is the ultimate initial trigger of mitosis, in which case only the initiation of S phase itself has to be accounted for and explained.

Competing interests

The author(s) declare that they have no competing interests.

Acknowledgements

Grant MCB-0323346 from the National Science Foundation supported this work. This research is supported (in part) by the National Institutes of Health through the University of Michigan’s Cancer Center Support Grant (5 P30 CA46592). Alexandra Cooper was invaluable as an editor of this paper. The PCR analysis was performed in collaboration with Joseph Washburn of the University of Michigan Microarray Core. Cells produced by automated membrane-elution are available for other laboratories to analyze proteins during the cell cycle.

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