RESEARCH ARTICLE

Schizosaccharomyces pombe grows exponentially during the division cycle with no rate change points

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Abstract

Length measurements during the division cycle of 86 individual *Schizosaccharomyces pombe* cells demonstrate that length grows exponentially with no change in the growth rate and no rate change point (RCP) observed for any cell. These results support the proposal that length extension, or cell growth, is exponential during the division cycle. The finding of exponential growth during the cell cycle is significant because these results challenge and contradict the current, consensus, widely believed, and widely accepted view that growth of *S. pombe* during the division cycle is complex with ranges of linear growth changing at proposed RCPs. Biochemical synthetic patterns support and explain the observed exponential cell growth. Exponential growth of *S. pombe* is consistent with, and supports, the central tenets of the continuum model.

Introduction

Mitchison & Nurse (1985) proposed that length growth of *Schizosaccharomyces pombe* is complex with linear growth regions separated by rate change points (RCPs). This pattern was termed 'bilinear', with an RCP occurring approximately one-third of the way through the cell cycle. Since that initial proposal, there have been numerous papers supporting a complex growth pattern for *S. pombe* (Kubitschek & Clay, 1986; Miyata *et al.*, 1988; Sveiczer *et al.*, 1996; Buchwald & Sveiczer, 2006; Baumgartner & Tolic-Norrelykke, 2009; Navarro *et al.*, 2012). Other studies by Mitchison (1957) and Mitchison *et al.* (1998) proposed that mass growth was linear with volume following a different and more complex pattern. It has been proposed that wild-type *S. pombe* grows linearly in mass with a more complex pattern of surface growth and with a mutant cell showing bilinear mass increase with a complex pattern of surface growth (Rappaz *et al.*, 2009). A recent paper reanalyzed films of growth from 1996 studies by Murdoch Mitchison and proposed that there is bilinear growth with a smooth, rather than abrupt, transition between the linear growth phases (Horvath *et al.*, 2013).

In contrast to these proposals, the growth of *S. pombe* has been shown to be simply exponential during the division cycle based on a replotting of published data (Cooper, 1998). However, that analysis was limited because it was based on measurements of the growth of one cell. More generally, it has been proposed as a general principle that exponential growth during the division cycle is valid for all cells (Cooper, 1979, 1981, 1987, 1988a, b, c; Cooper, 1990, 1991, 1998, 2001, 2004, 2006, 2012; Cooper *et al.*, 2009). The clearest example of this principle is the demonstration using differential analysis that *E. coli* grows exponentially during the division cycle (Cooper, 1988a, b, c, 1991).

This paper now presents data from a large number of cells – 86 to be precise – demonstrating that growth of *S. pombe* is exponential during the division cycle with no observable RCPs.

Results

Plotting of original cell length data for *S. pombe* growth

Length measurements during the division cycle of *S. pombe* were obtained from two laboratories. Stephan

Baumgartner sent the raw data (Baumgartner & Tolic-Norrelykke, 2009) for cells growing at three different temperatures (25 °C, 24 cells; 28 °C, 20 cells; and 32 °C, 40 cells). Supplementing these measurements are the length growth data for a wild-type *S. pombe* and a *wee* (smaller size) mutant obtained directly from the published paper of Buchwald & Sveiczer (2006), which were in turn obtained originally as described in two papers (Sveiczer *et al.*, 1996; Mitchison *et al.*, 1998). Thus, the growth patterns of 86 cells during the division cycle were analyzed.

The results of Stephan Baumgärtner and Iva M. Tolic-Nørrelykke were originally presented as the mean values obtained by averaging the values for a large number of different cells at different time points during the cell cycle. The cell sizes from different cells at different times were averaged, and the average growth pattern was plotted. It was the analysis of the averaged data that led to their conclusion that growth of *S. pombe* is bilinear.

The original data for individual cells are shown in Figs 1–3. The original averaged data line graphs are shown as the uppermost, thicker, lines in Figs 1–3. These averaged lines were used to support bilinear growth (Baumgartner & Tolic-Norrelykke, 2009). The results are plotted on a semi-logarithmic scale. A cell growing exponentially during the division cycle will give a straight line on a semi-logarithmic plot. As can be seen from Figs 1–3, the lines for individual cells are straight with no observable break point, nor any suggestion of any change in rate. In Figs 1 and 3, the short arrow notes the RCP time reported for the aggregated cells (Baumgartner & Tolic-Norrelykke, 2009). The plotted lines for individual cells in Figs 1–3 indicate that there is no RCP observed in any of the length growth patterns.

Similar results are found for the data of Buchwald & Sveiczer (2006) as shown in Fig. 4. The results in Fig. 4 are for two single cells. No break point is observed. The data fit a straight line indicating exponential growth during the division cycle. A trend line is superimposed on the data. Statistical analysis (Excel spreadsheet) gives R^2 values of 0.99 and 0.98 showing the data are consistent with exponential growth. The two arrows in Fig. 4 show where Buchwald and Sveiczer indicated that they observed a break point or RCP.

Statistical analysis of growth patterns

A summary statistical analysis plotting of the R^2 values of the 86 lines in Figs 1–4 is shown in Fig. 5 where the R^2 values are shown in a histogram to indicate the strong fit of all of the data to an exponential pattern. A value of 1.0 is a perfect fit, so values close to 0.99 and 0.98 are indicative of a pattern very close to or statistically indistinguishable from exponential growth.

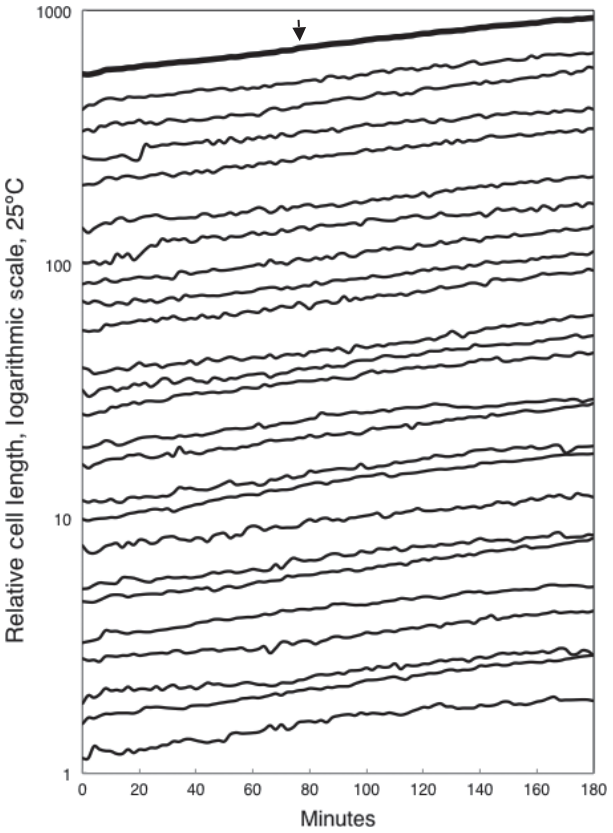

Fig. 1. Growth of *Schizosaccharomyces pombe* at 25 °C [From Baumgartner & Tolic-Norrelykke (2009)]. The original measurements of individual cell lengths were generously sent by Stephan Baumgartner. The data for individual cells were adjusted by multiplying the data for each cell by a constant factor, so the individual lines are visible. Without this adjustment, all the lines would lie close together and would not be seen as individual lines. The top line (thicker) is the mean value of all the lower graphs and was the line analyzed by Baumgartner & Tolic-Norrelykke (2009). The arrow at the top notes the time (59 min) at which the RCP is reported to exist by Baumgartner and Tolic-Norrelykke. The measured doubling time of the averaged line is 222 min.

In an additional test of the bilinear model, Baumgartner and Tolic′–Nørrelykke took the differences between different points for averages in Figs 1 and 3, and the results are shown in Fig. 6 (taken directly from their paper). The two horizontal lines are their fit to the data with a break point between the two linear growth regions that they postulated to exist. The lines are horizontal because the MATLAB program used to draw the two lines was constrained to have a zero slope (S. Baumgartner, pers. commun.). A thicker line has been added, drawn by eye, to indicate that a plausible fit to the data is a line with a positive slope, indicating increasing rate of length growth as the cell progresses through the cell cycle. This thick line is consistent with exponential growth, as the

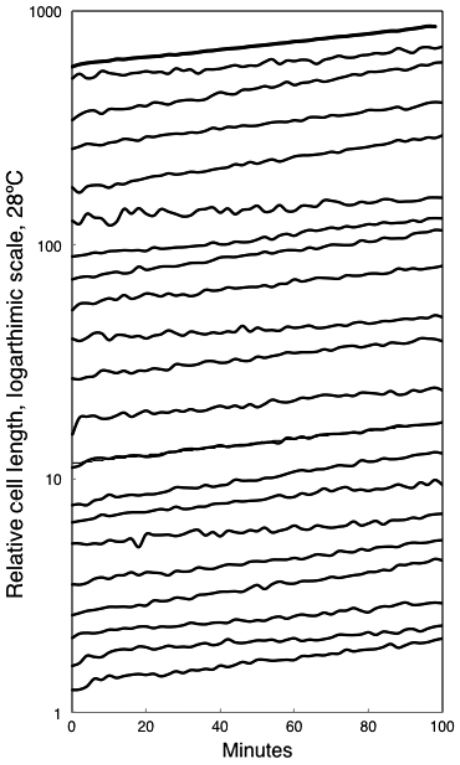

Fig. 2. Growth of *Schizosaccharomyces pombe* at 28 °C. As in Fig. 1, but a different temperature. The data were sent by Stephan Baumgartner (Baumgartner & Tolic-Norrelykke, 2009). The doubling time of these cells using the average graph is 232 min.

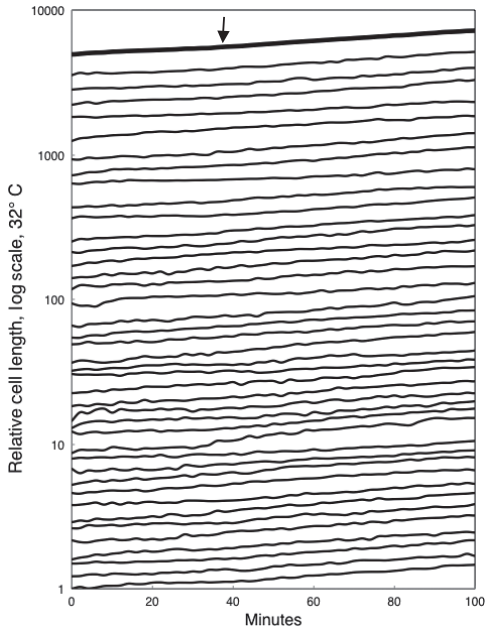

Fig. 3. Growth of *Schizosaccharomyces pombe* at 32 °C. As in Fig. 1, but at a different temperature. The data were sent by Stephen Baumgartner (Baumgartner & Tolic-Norrelykke, 2009). The arrow at the top is the time (39 min) at which the RCP is reported to exist by Baumgartner and Tolic-Norrelykke. The doubling time of these cells using the average graph is 181 min.

absolute increase in length per time period would be expected to increase over the division cycle.

Searching for RCPs and bilinear growth

If growth were bilinear and there were actually a change in growth rates at a particular RCP and if that RCP were biologically meaningful, functional, and real, one would expect that at least one individual cell of the 86 plotted in Figs 1–4 would show a clear rate change point. No cells in Figs 1–4 exhibit a rate change point.

At this time, it is not clear how to fit the individual cell data to a bilinear pattern because it is not obvious where one would put the break point for each line. Without the break point, one cannot determine where either of the two linear phases begins and ends.

Discussion

Models and mechanisms of cell growth

It is proposed here that cell growth is primarily due to the exponential increase in the mass of the cell (Cooper, 1988a, b, c). The total mass growth of a cell is the sum of the increase in each of the components of the cell. Because the cytoplasm (ribosomes, enzymes, etc.) is the dominant portion of cell mass, the growth of the cell is very similar to the pattern of cytoplasm increase (Cooper, 1988a, b, c). It has been shown, in *E. coli* (Cooper, 1988a, b, c), that the cytoplasm, primarily ribosomes and other proteins), makes more cytoplasm in proportion to the existing mass. This leads to exponential increase in cell mass during the division cycle. If there were to be a sudden change in the rate of mass accumulation, a signal would have to propagate over an enormous number of ribosomes, RNA polymerases, and other functioning enzymes to produce a sudden change in the rate of mass increase. Exponential growth does not need that rate change biochemistry.

Applying the idea that mass increase is the agent that produces the increase in cell surface, it is proposed that the exponential increase in mass in *S. pombe* is the determinant for length increase. Simply put, the exponential increase in mass leads to the exponential increase in cell length.

Apropos the biochemical question of a possible trigger in *S. pombe* for a rate change point, this proposal was tested by adding hydroxyurea, an inhibitor of DNA synthesis, to growing cells. It was reported (Baumgartner & Tolic-Norrelykke, 2009) that adding hydroxyurea led to the disappearance of a break in the growth pattern. With

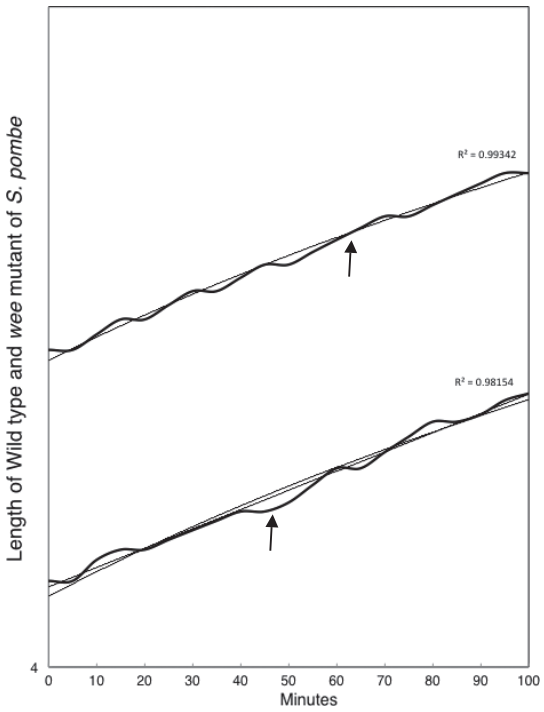

Fig. 4. Growth of *Schizosaccharomyces pombe*, data of Buchwald & Sveiczer (2006). Two individual cells were analyzed, a wild-type and a *wee* mutant. A trend line (Excel) has been added to each of the data lines and is the thin, straight line through the data points. The R^2 values are indicated showing that the data fit an exponential function. The upper line is the wild-type, and the lower line is the *wee* mutant. The measured doubling time of the wild-type cell is 160 min and of the wee mutant is 151 min.

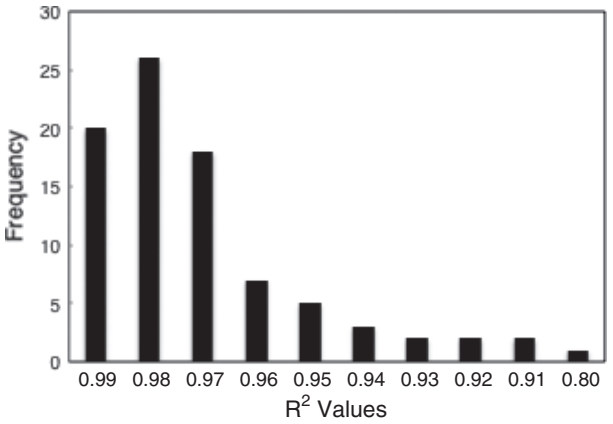

Fig. 5. Statistical analysis of cell growth during the division cycle. The R^2 values for exponential growth for each of the 86 cells in Figs 1–4 were determined and are presented as a frequency graph of all of the results. Most of the lines have an R^2 of 0.99 or 0.98, indicating that the lines are statistically close to exponential and cannot be distinguished from exponential.

hydroxyurea, no break point could be observed. Their conclusion was that something about the S phase or initiation of DNA synthesis was related to the proposed RCP. However, this conclusion is only valid if there is an RCP in the untreated cultures. To use the hydroxyurea experiment to prove that a rate change point is related to some particular cell cycle event such as DNA replication therefore is not a valid conclusion. Only if there is an RCP in untreated cultures that disappears when hydroxyurea is added can one use the hydroxyurea experiment to conclude anything about the effect of inhibiting DNA replication.

Mechanistic problems with bilinear growth

In contrast to the absence of 'mechanism' for producing exponential growth, the proposal of bilinear growth has a number of problems. Linear growth means that the new cytoplasm made by an extant amount of cytoplasm does not engage in new cytoplasmic growth. Linear growth thus implies that new cytoplasm is treated differently from pre-existing cytoplasm, and at some instant (the RCP), this newly made cytoplasm is activated to begin producing new cytoplasm. It is difficult to imagine how an enormous number of ribosomes, RNA polymerases, and other cellular elements can be activated at some point during the cell cycle.

A deeper problem with the proposal of an RCP is that this model means that prior to the RCP, the cell was not growing as fast as it could because it did not utilize newly made cytoplasm. From an evolutionary viewpoint, this is deleterious to the cell as a cell that activated its newly made cytoplasm immediately would grow faster and produce the exponential growth proposed here.

The relationship between mass and surface growth during the cell cycle

It is of interest to consider the alternative view where mass growth is not the determinant of surface growth and the two cell elements grow independent of each other, as expressed in a recent review (Marguerat & Bahler, 2012):

> Thus, as cells grow, they generally need to synthesize more proteins to maintain the appropriate concentration of these molecules.

The idea expressed by this quote is that the increase in cell surface is independent of cell mass increase. Thus, a cell surface can grow in some pattern, and the cytoplasm will then increase to accommodate the volume produced by the increase in cell surface. For example, an *S. pombe* cell surface can grow with a break point, and the mass will then be synthesized to fill up the space provided by the increase in cell volume.

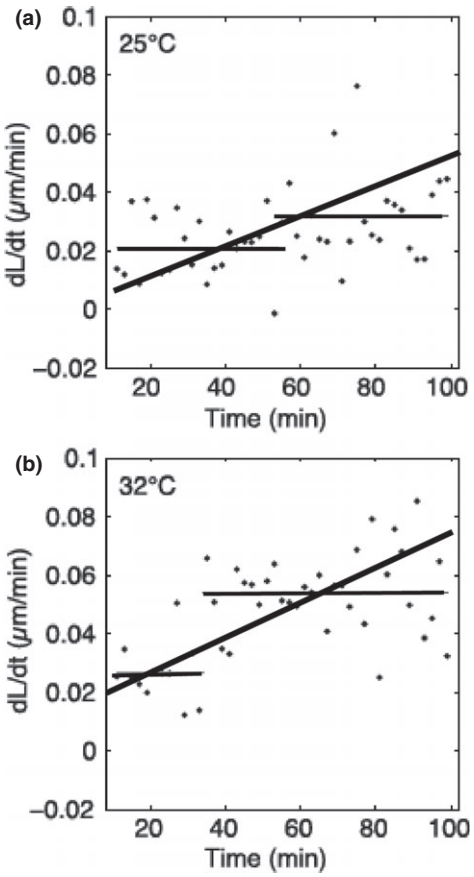

Fig. 6. Differential analysis of cell growth. Replotting of the differential analysis from Fig. 6 of (Baumgartner & Tolic-Norrelykke, 2009). The points are calculated from the difference between different length measurements for the average data for cells at 25 °C (panel a, top) and at 32 °C (panel b, bottom). The horizontal lines are those of Baumgartner and Tolic-Norrelykke and the thicker line is one drawn by eye through all the data points. A computer plotting of the two regions of the horizontal lines (not shown) indicates that the points at the earlier times have a negative slope while the later times have a positive slope.

The exponential model proposed here is that the increase in cytoplasm is the agent that causes and produces the surface increase. Surface does not grow independent of cytoplasm or cell mass increase. Mass growth determines surface and volume growth, and the two are inextricably linked.

The relationship between mass increase (the sum of cytoplasm, genome, etc.) and surface growth may be encapsulated in a metaphorical image. Consider a sausage-shaped balloon where air is being pumped continuously into the balloon. As more air enters the balloon and there is no increase in the surface area of the cell, the pressure on the balloon's inner surface increases. Now imagine that additional rubber is added to the balloon surface to just allow the increase in volume with no increase in pressure as the newly added rubber accommodates the increased air pressure. Similarly, in a cell, the increase in mass leads to tension on the surface that leads to surface increase. This model is clearly understood in bacteria where the peptidoglycan structure in *E. coli* will lead to the insertion of new material as cytoplasm grows (Cooper, 1989, 1991). Further, it is a classic observation that inhibiting surface synthesis without inhibiting mass increase leads to the eventual bursting of the bacterial cells.

Deciding between different models of *S. pombe* growth

How does one choose between different growth models when the fit of data to each model is quite close? It is widely accepted that it is difficult to distinguish between a linear pattern and an exponential pattern (Cooper, 1988a, b, c, 2006) and even more so to distinguish between a bilinear pattern and exponential increase over the cell cycle where the mass increases only a factor of two. This is shown in Fig. 7. Those who propose a bilinear pattern have used statistical analysis of the experimental points to propose that growth is bilinear (Buchwald & Sveiczer, 2006; Baumgartner & Tolic-Norrelykke, 2009). Statistical analysis cannot actually distinguish between exponential and bilinear growth with simple measurements of cell lengths. Statistical analysis cannot show that bilinear growth is the growth pattern for *S. pombe*. If there were even one of the 86 lines analyzed in Figs 1–4 that showed a break point, or any indication of a rate change, one might suggest some functionality for an RCP. Looking at the data in Figs 1–4, the lines are straight with no visible bend or break, and therefore, growth is simply exponential during the cell cycle. Because integral measurements cannot decide between bilinear and exponential patterns to the satisfaction of all who study this problem, let us now look at an approach to the problem to decide which model of cell growth is correct.

Differential measurements of cell growth as a deciding approach

There is an experimental approach that can distinguish between the different proposals of *S. pombe* growth. Consider a culture grown for many generations with a radioactive label such as C14-leucine. After many generations, the amount of label per cell would be proportional to the total mass of the cell. Now add, for a short period of time, a different label such as tritiated (H3) leucine. This second label would be incorporated in proportion to the mass synthesis at that short labeling period. Now fix the cells, wash the cells, and separate out the cells by some method that distinguishes between cells of different cell cycle ages. For

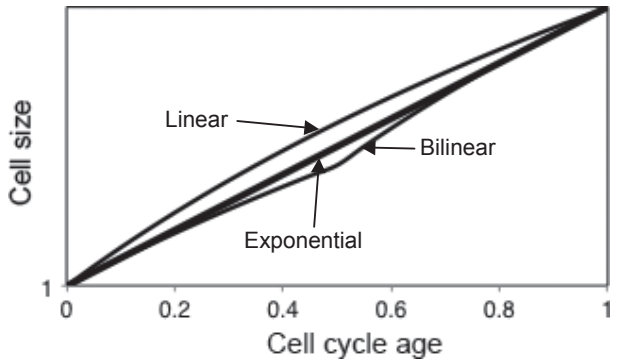

Fig. 7. Comparison of exponential, linear, and bilinear patterns of growth when plotted on a linear ordinate or a logarithmic ordinate. With 20 points analyzed, the exponential R^2 is 1.000, the linear is 0.99137, and the bilinear is 0.99545.

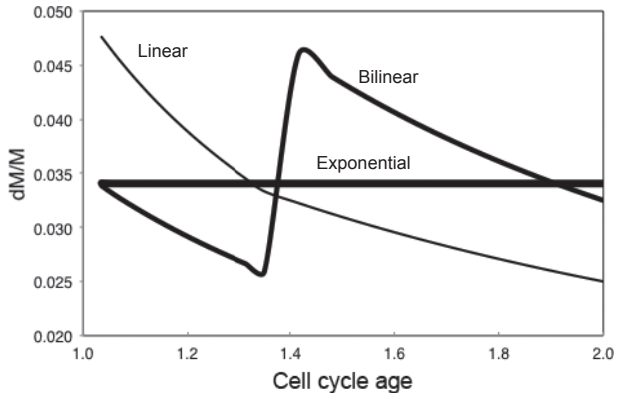

Fig. 8. Expectations for 'differential' analysis of cell growth during the division cycle. A differential analysis measures not the amount of mass or cell length at various times, but the change between different points. The plot is the differential amount per extant amount of material at different times. The horizontal line is for exponential growth as the amount of material made in any short period of time is directly proportional to the extant amount, so the ratio (dM/M, where M is mass) is constant. The linear and bilinear lines are the expectations for those patterns of growth. This analysis was the basis for the experiments that demonstrated that *Saccharomyces cerevisiae* growth was clearly exponential (Elliott & McLaughlin, 1978).

example, one can use hydrodynamic separation in a sucrose gradient where the larger (older) cells will be preferentially at the bottom of the tube. Or one could use the baby machine approach that worked for *E. coli* and has been applied to yeast cells (Helmstetter, 1991). The exponential model would predict, as shown in Fig. 8, that the ratio of tritium to C14 label would be constant over the range of cell cycle ages. The other models would give decidedly different results as shown in Fig. 8. Or even more simply, one could do an experiment similar to that done for *E. coli* where pulse labeled cells are separated by age using the membrane elution method (Helmstetter, 1991) and get a clear decision between exponential and linear growth patterns (Cooper, 1988a, b, c).

In fact, this experiment has been performed on *Saccharomyces cerevisiae* with results that unambiguously confirmed exponential growth of the cell during the division cycle (Elliott & McLaughlin, 1978).

There is an interesting and important historical precedent for the analysis presented here. The growth of *E. coli* had been proposed to be linear during the division cycle (Kubitschek, 1967a, b, 1968, 1969, 1970, 1981, 1986). It was difficult to distinguish between the linear and exponential proposals using 'integral' measurements such as measuring cell sizes or lengths during the division cycle. See Fig. 3 in (Cooper, 1988a, b, c) as an example of this problem.

However, when a 'differential' measurement was used, where the difference between linear and exponential is absolutely clear as shown in Fig. 2 of (Cooper, 1988a, b, c), the result was quite clear that growth of *E. coli* was exponential and not linear (Cooper, 1988a, b, c, 2006).

On plotting growth data

Perhaps, one of the most important lessons to be derived from the controversy over the growth pattern of *S. pombe* is how data should be plotted. Those papers proposing bilinear growth used a rectangular plot, with a linear ordinate. When exponentially growing cells are plotted on this type of graph, the curved result allows one to see, or imagine, straight lines in the data. By plotting the data on using a logarithmic ordinate, one can clearly see that all the data can fit an exponential pattern of growth, as shown here in Figs 1–4.

A fundamental misunderstanding

There is an idea related to the controversy over bilinear and exponential growth during the division cycle that must be clarified. It is clearly presented in a recent paper on the subject (Horvath *et al.*, 2013) where it is written:

> The time profile of size increase is a fundamental problem as linear growth is thought to support homeostasis, whereas exponential growth is rather thought to operate against it. In the latter case, more stringent control mechanisms are required to maintain constancy of cell size.

It is incorrect to state that exponential growth, in some way, would lead to lack of homeostasis of cell size and in some way would require more stringent control mechanisms to maintain constancy of cell size. It has been clearly demonstrated (Cooper, 2006) that any mode of growth (linear, bilinear, exponential) will maintain size constancy with the presence of a size-determined signal

for control of cell cycle events. In the case of the continuum model, it has been proposed that the size at initiation of DNA replication is the controlling element, but it is irrelevant what the size signal affects. The main point is that exponential growth patterns clearly can produce size homeostasis.

The continuum model for progression through the cell cycle

The current, dominant, consensus, and widely accepted view of cell cycle progression is that during the cell cycle, different genes are expressed at different times. The RCP model for *S. pombe* fits into this viewpoint of cell cycle progression, as the RCP is a cell cycle event that occurs at a particular time during the cell cycle.

An alternative view, the continuum model, proposes that the cyclic expression of genes does not occur and that growth during the cell cycle is basically uneventful. The importance of the conclusion, presented here, regarding the absence of the RCP in *S. pombe* is that this result supports and is consistent with the continuum model of cell growth during the division cycle. The continuum model postulates that there are no major events (other than initiation of DNA replication) during the division cycle of unperturbed cells (Cooper, 1981, 1982, 1988a, b, c, 2000, 2012; Shedden & Cooper, 2002).

An analogous reconsideration of published data is found in the reanalysis of the data proposing cell cycle-specific gene expression patterns (Cho *et al.*, 2001) showing that such a proposal was based on unsynchronized cells, irreproducible data, and results that were consistent with random statistical variation (Shedden & Cooper, 2002).

While there have been proposals that yeast cells express many different genes at different times during the division cycle using microarray analysis to measure mRNA production (Spellman *et al.*, 1998; Rustici *et al.*, 2004; Oliva *et al.*, 2005; Peng *et al.*, 2005), a 1978 paper demonstrated unambiguously that 150 proteins that were studied did not vary at all during the division cycle (Elliott & McLaughlin, 1978). This result is consistent with the proposal that even if mRNAs varied during the division cycle, their impact on protein variation during the division cycle would be negligible (Cooper & Shedden, 2007).

On choosing between different models and the evolutionary imperative

How does one choose between the proposed exponential and bilinear (or other nonexponential) patterns of growth during the cell cycle? I suggest three reasons to choose the exponential pattern.

The first reason is the data. As shown here in Figs 1–4, the data strongly support exponential growth. The bilinear patterns are based on a flawed graphing approach (linear rather than logarithmic scales), and when properly plotted, the data support exponential growth.

Second is the biochemical basis of cell growth. All proposed linear growth patterns mean that new cytoplasm does not get activated to create new cytoplasm as the cell grows. Thus, the cell is not growing as fast as it could if the new cytoplasm joined in synthesis as soon as it was made. In the bilinear growth pattern, just prior to the transition to the second linear phase, the cell is not growing as fast as it could because at the transition the cell quickly grows at a faster rate. What is most troubling about the postulation of a rate change point is that in none of the papers that have proposed a bilinear pattern have any mechanistic, biochemical, or biological mechanism been suggested to explain this change in growth rate. Simply put, how does the cell suddenly activate the large number of ribosomes, RNA polymerases, and other cellular elements to change to a new growth rate? Until some plausible, believable, and understandable mechanism is proposed to explain nonexponential growth, the bilinear modes of growth should be discarded.

In contrast, the exponential model has new cytoplasm joining in to synthesize new cytoplasm as soon as it is made. There is no 'mechanism' controlling exponential growth as exponential growth is inherent in the way cytoplasm is made and the way cytoplasm makes new cytoplasm.

Third and finally, the exponential pattern fits the evolutionary imperative that a cell should grow as fast as possible to make as many descendents as possible over time. For a cell to not use its cytoplasm as efficiently as possible to grow as quickly as possible is antievolutionary, and this should be considered when choosing between different models.

In summary, I propose that the data, the biochemistry, and the logic of cell growth imply that cells grow exponentially during the division cycle.

More to the point, in all the papers proposing nonexponential models, I have not read one proposal that is believable, acceptable, and biologically or biochemically understandable that explains how there is a sudden or even slow change in growth rate between linear phases of growth. Until such an explanation is given, one must be cautious and skeptical regarding acceptance of linear-based models.

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