



" ! ... Comment ... ? "



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Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others. In other instances we may have a useful piece of methodology that we would like to share.

The Editors hope that readers will take full advantage of this section and use it to raise matters that hitherto have been confined to a limited audience.

Jon Saunders
Editor-in-Chief

Length extension in growing yeast: is growth exponential? – Yes

Entia non sunt multiplicanda praeter necessitatem
William of Occam

Exponential cell growth has been proposed as the most general pattern of cell mass increase during the division cycle for both prokaryotes and eukaryotes (3). Experiments on the differential rate of synthesis during the division cycle using the membrane-elution method (1) clearly demonstrate exponential growth during the bacterial division cycle. A general growth law for bacteria during the division cycle has been presented (2).

Do eukaryotic cells such as yeast also have an exponential pattern for cell mass increase? Recent measurements on the growth of single cells of wild-type *Schizosaccharomyces pombe* have been used to support the idea that these cells grow linearly, with a change in the rate of linear growth occurring at a particular point (the rate change point, or RCP) in the division cycle (12). The RCP is put forward as a possible regulatory point for control of the cell cycle.

The RCP was originally proposed on the

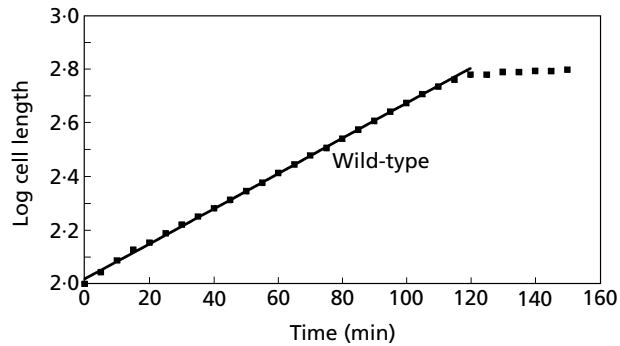


Fig. 1. Growth in length of a single wild-type cell of *S. pombe*. The data for the cell lengths from Fig. 2 of Svecizer *et al.* (12) are plotted on a semi-logarithmic scale. The data are indicated by the filled squares. The straight line drawn through the points is the best fit based on a minimization of deviation of points from the straight line. A straight line on semi-logarithmic coordinates indicates exponential growth.

basis of enzyme measurements on synchronized cells by Mitchison & Creanor (10). The model based on enzyme changes was then extended to cell growth by Mitchison & Nurse (11). Appended to the paper by Mitchison & Creanor (10) is a statistical appendix by D. A. Williams, in which the data are subjected to statistical tests in order to support the idea that the data fit linear segments better than an exponential model.

Because the postulation of an RCP between linear growth segments does not fit with the proposal of a general model of exponential cell growth, I have reanalysed the original data of Svecizer *et al.* (12) and show that the data are consistent with, and furthermore strongly support, exponential growth of *S. pombe* during the division cycle.

The original data on cell-size measurements were kindly sent to me by e-mail by Dr Bela Novak. The original data of Svecizer *et al.* (12) were replotted using semi-logarithmic coordinates (Fig. 1). Plotting exponential growth on linear coordinates, as was done in the original publication, gives an upwardly curving line which may appear, to the eye, as due to two linear segments. As is shown in Fig. 1, the data for the wild-type *S. pombe* fit an exponential growth pattern extremely well. There is no need to invoke any change in growth pattern, nor is there any deviation from exponentiality until the very end of the cycle. The reason for the cessation of growth at the end of the cycle is not known.

Linear regression analysis was used to

compare the different models. The comparisons are listed in Table 1, where the r^2 values for different analyses are presented. An r^2 value of 1 means a perfect fit, and the higher the value the better the fit. Values above 0.9900 are essentially perfect fits to the data and are for all practical purposes indistinguishable. When the first 11 points (before the proposed RCP) are analysed for a linear fit, a good fit to a linear regression is obtained (case A), and the same is found for the second linear segment of 13 points after the RCP (case B). Since in each of these examples two para-

► GUIDELINES

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Table 1. Statistical comparison of linear and exponential models

Cases A–C were linear regressions of the original data on rectangular coordinates; case D was a linear regression of the logarithm of the original data, so that the fit to an exponential case was tested.

Case	Points analysed (no. of parameters)	Segment analysed	r ² value
A	11 (two)	First linear segment	0.99850
B	13 (two)	Second linear segment	0.99888
C	24 (three)	Two linear segment spline	0.99959
D	24 (two)	Single exponential	0.99935

meters are required (an origin and a slope for each line), the total number of parameters to get a fit to all of the data is four.

If a best fit to two linear segments with a single bilinear spline fit is analysed (case C), we find a very good fit as well, although in this case there are three parameters to the formula. These three parameters are the common midpoint value between the two linear segments, and the two slopes of the linear segments.

An analysis using all 24 points in the two linear segments and fitting them to a single exponential model gives an essentially indistinguishable fit (case D), although in this case there are only two parameters in the exponential model, a single origin and a single slope. Observe that the statistical fit for the two-parameter exponential model (case D) is even better than the fit to the two two-parameter linear models (cases A and B).

How does one distinguish between the different models? The numerical distinctions (r² values) between the different models are negligible. Therefore it is best to use the simplest model and this is obviously case D, where only two parameters are needed to fit all of the data. That the statistical differences between the models in Table 1 are negligible can be seen if one considers that a model with 46 parameters, taking each point as the start of a line segment, and having a slope going perfectly to the next point, would yield an r² value of 1.0000. Yet this model with a perfect fit would be excluded as being too complicated and arbitrary because of the large number of parameters used to get this perfect fit. Simplicity considerations (Occam's Razor) suggest that the two-parameter model that accounts with a single formula for all of the points is to be preferred over more complex models (more parameters). The visual indication that growth is exponential (Fig. 1) is supported by the more precise statistical analysis (Table 1).

I suggest that the simplest explanation for cell growth in all cells is exponential growth during the division cycle (3). One may believe, if one wishes, the more complex RCP model. However, in order to do this one must note that the data fit an alternative model equally well, the alternative model is simpler, and the theoretical analysis of cellular growth is strongly consistent with the exponential growth model. The exponential growth

model is based on a very simple and biochemically sound explanation for exponential growth (3).

The linear growth model has a long history. Using interferometry on single cells, Mitchison proposed linear growth in dry mass in fission yeast (7) and in budding yeast (8). The same technique was also used on *Streptococcus* (9), where declining rate curves were found.

The problem with many of these measurements of mass growth is that they are 'integral' measurements (i.e. they measure the total amount of something at sequential time-points) rather than 'differential' measurements (which measure the change in rate of synthesis of some cell component at different time-points). A bilinear or linear model is difficult to distinguish from exponential growth using an integral measurement (the greatest expected deviation will be 6 % between the theoretical curves). Differential measurements on the same data produce significant differences (exponential growth predicts an exponential pattern in the rate of synthesis, while the linear model proposes a constant rate of synthesis during a linear period).

A general linear growth model was put forward by Kubitschek (6). In retrospect, it is now known that the experimental basis for this proposal (4, 5) is flawed. Some of the uptake determinations (specifically thymidine) did not accord with what was subsequently shown to be the distinctly non-linear pattern of thymidine incorporation and DNA synthesis (3). Thus, the experimental support for linear growth, i.e. a constancy of uptake of all substances studied, is not valid.

Whether the length of a cell reflects biomass or not is not relevant to this analysis. The question raised here is whether it is justifiable to use published length measurements to support linear growth segments (in cell length) separated by an RCP. The data clearly fit exponential kinetics equally well.

The argument can be made that growth of eukaryotic cells is more complex than that of prokaryotic cells, transcription rates and translation rates are known to vary substantially and cell cycle controls are many and various and thus more complex patterns are congruent with what has been discovered over the last decade. However, this suggestion

is not necessarily true. A general critique of the entire notion of complex cell cycle controls has been published (3). The point of the arguments presented here is that one should not use suggested complex controls in eukaryotes to support the RCP model, which is now used to support complex cell cycle controls, for to do so would be to use a circular argument. A similar argument is that data for eukaryotic cells should be considered on their own merits (i.e. without reference to bacterial results) because the mechanics of the eukaryotic cell are more complex. This is precisely what has been done here. Does an RCP exist which is an indication of increased complexity in eukaryotic cells? One should not assume complexity and then use that to justify the quite weak evidence for an RCP.

The data on yeast cell growth presented here are strongly supportive of exponential growth between divisions. No rate changes between linear growth segments need be postulated as controlling elements in the cell cycle. The data fit the proposal of a general pattern of simple, exponential, mass synthesis during the division cycle. This analysis shows there is no compelling reason to accept the linear model of cell growth during the division cycle of *S. pombe*. In contrast, the data fit an exponential pattern for cell growth during the division cycle.

Dr Bela Novak was extremely kind in supplying the original smoothed data for reanalysis. Dr Eduard D. Rothman of the University of Michigan Center for Statistical Consultation and Research gave invaluable help on the regression analysis presented in this paper.

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- Cooper, S. (1988a). Leucine uptake and protein synthesis are exponential during the division cycle of *Escherichia coli* B/r. *J Bacteriol* 170, 436–438.
- Cooper, S. (1988b). What is the bacterial growth law during the division cycle? *J Bacteriol* 170, 5001–5005.
- Cooper, S. (1991). *Bacterial Growth and Division, Biochemistry and Regulation of Prokaryotic and Eukaryotic Division Cycles*. San Diego, CA: Academic Press.
- Kubitschek, H.E. (1968a). Growth during the bacterial cell cycle: analysis of cell size distribution. *Biophys J* 9, 792–809.
- Kubitschek, H.E. (1968b). Constancy of uptake during the cell cycle in *Escherichia coli*. *Biophys J* 8, 1401–1412.
- Kubitschek, H.E. (1970). Evidence for the linearity of linear cell growth. *J Theor Biol* 28, 15–29.
- Mitchison, J.M. (1957). The growth of single cells. I. *Schizosaccharomyces pombe*. *Exp Cell Res* 13, 244–262.
- Mitchison, J.M. (1958). The growth of single cells. II. *Saccharomyces cerevisiae*. *Exp Cell Res* 15, 214–221.
- Mitchison, J.M. (1961). The growth of single cells. III. *Streptococcus faecalis*. *Exp Cell Res* 22, 208–225.
- Mitchison, J.M. & Creanor, J. (1969). Linear synthesis of sucrose and phosphatases during the cell cycle of *Schizosaccharomyces pombe*. *J Cell Sci* 5,

373–391.

11. Mitchison, J.M. & Nurse, P. (1985). Growth in cell length in the fission yeast *Schizosaccharomyces pombe*. *J Cell Sci* 75, 357–376.

12. Svecizer, A., Novak, B. & Mitchison, J.M. (1996). The size control of fission yeast revisited. *J Cell Sci* 109, 2947–2957.

Authors' reply

Length growth in fission yeast: is growth exponential? – No

Despite the assertion by Cooper (2) that length growth is exponential, his Fig. 1 shows this to be untrue. It has been known for many years (4) that growth in length in fission yeast ceases for about the last 20 % of the cell cycle and it is usually assumed that this is because the capacity for wall synthesis is concentrated on forming the septum. It is not, however, a period when bulk growth stops since increases continue in dry mass, protein and RNA (4). However, there is a marked change in the rate of wall extension at the beginning of this period.

There is another more subtle change in the rate of extension which Cooper (2) has also ignored in his advocacy of a simple exponential growth pattern. It is clear from his Comment and his earlier book (1) that what he has in mind is an exponential pattern in which the rate doubles over the cycle. In this case, there are no sharp rate change points (RCPs) either during the cell cycle or at division when one cell becomes two daughter cells. However, in wild-type fission yeast, the rate only increases by an average of about 30 % through the growing period of interphase (6). To maintain balanced growth, the rate of the system as a whole must increase by another 70 % at division. Only then will each daughter cell have the same growth pattern as its mother cell. The existence of the rate change at division has been shown in the following way (unpublished measurements). The sum of the initial growth rates of two daughter cells after a division was compared to the growth rate of their mother cell just before its constant length period. There was a rate increase in four cases out of five of the wild-type cells examined. After a block and release experiment with *cdc2*, the population is not in balanced growth and a rate change during the growth period cannot be observed in these oversized cells (6). However, using the method above showed a rate change at division in six out of eight cases examined. So length growth is not exponential and there are two marked rate changes. Nor is it simply a matter of switching growth off and on since the rate change at division involves a marked acceleration of the system.

There remains the problem of the third rate change during the interphase growth

period. It is not easy, considering the scatter in length measurements, to be certain of two linear segments, with a rate change which averages only 30 % and can be less (4, 6). The linear regression on a semi-logarithmic plot used by Cooper (2) is not sufficiently sensitive, so we have used the much more sensitive measure of the rates of length growth. The difference between successive length measurements was taken from the unsmoothed data and these differences were then smoothed by the 'rsmooth' command of the Minitab program. One result is given in Fig. 1 with the length measurements and the smoothed rates. The rate pattern is clearly one that would be given by two linear segments with a rate change of about 30 %, though the sharpness of the step rise will be somewhat diminished by the smoothing process. It is quite different from exponential growth where the rate should increase steadily throughout the growth period. So here is a cell which certainly does not grow exponentially. In other cells which we have examined, the pattern is less clear. There is a step at the RCP but there may also be other rate changes before and after this point which vary with the exact points at which the growth period starts and stops. These are not regular in their appearance and pattern, and occur because of the high sensitivity of the analysis on data that are limited by slight changes in focus and by limited resolution of the optics and of the measurements on projected photographic images.

This degree of variation makes it impossible to use a formal statistical test between two simple models of linear versus exponential growth. However, we have seen no cell showing simple exponential growth. Estimation of the RCP by eye is surprisingly effective since the eye carries on a smoothing process over minor changes. It is worth mentioning that the growth curves for *wee1* mutants have a much more conspicuous interphase rate change of 100 % and no rate

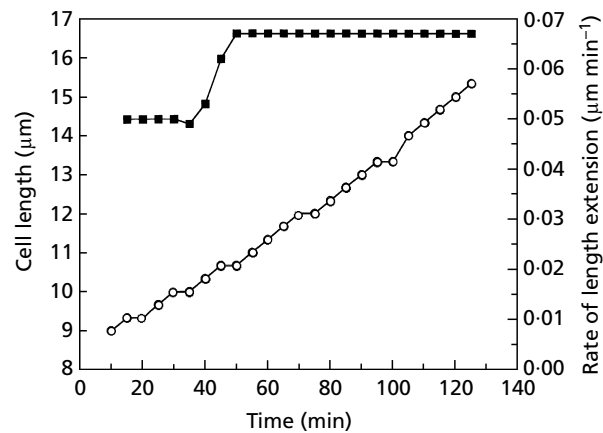


Fig. 1. Length extension in the growing period of a single wild-type cell of *Schizosaccharomyces pombe*. Lower curve, cell length; upper curve, smoothed rate of extension (see text).

change at division (6). The existence of this large rate change was demonstrated clearly by our new method of generating difference patterns. It seems most unlikely that the elimination of the *wee1* gene product causes a change from exponential interphase growth to two linear segments.

It is all very well to trot out old Father William (of Occam), but few people will believe that his famous 'Razor' is a suitable tool to shape all cell growth to the same simple exponential. It may be that the various bulk parameters of growth have the same exponential pattern in *Escherichia coli*, but this is not so in some other eukaryotic cells. In fission yeast, the growth patterns for volume, dry mass and total protein are all different from each other (4) and the same is true in budding yeast. None of these patterns in fission yeast is a simple exponential. Moving to different cells, growth curves with falling rates through the cycle (the opposite of an exponential) have been found in reduced weight (= dry mass) in *Amoeba proteus* (5) and in dry mass and volume in *Streptococcus faecalis* (3).

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1. Cooper, S. (1991). *Bacterial Growth and Division, Biochemistry and Regulation of Prokaryotic and Eukaryotic Division Cycles*. San Diego, CA: Academic Press.

2. Cooper, S. (1998). Length extension in growing yeast:

is growth exponential? – Yes. *Microbiology* 144, 263–265.

3. Mitchison, J.M. (1961). The growth of single cells. III. *Streptococcus faecalis*. *Exp Cell Res* 22, 208–225.

4. Mitchison, J.M. (1989). Cell cycle growth and periodicities. In *Molecular Biology of the Fission Yeast*, pp. 205–242. Edited by A. Nasim, P. Young & B.F. Johnson. San Diego: Academic Press.

5. Prescott, D.M. (1955). Relations between cell growth and cell division. I. Reduced weight, cell volume, protein content and nuclear volume of *Amoeba proteus* from division to division. *Exp Cell Res* 9, 328–337.

6. Sveiczler, A., Novak, B. & Mitchison, J.M. (1996). The size control of fission yeast revisited. *J Cell Sci* 109, 2947–2957.

Further correspondence

The central question asked here is “Do the data that were used to show two linear segments during the first 80 % of the division cycle also support exponential growth?” I can only reiterate, despite the assertion by J. M. Mitchison, A. Sveiczler & B. Novak (above) that my Fig. 1 definitely shows that length growth is exponential and not bilinear. *Res ipsa loquitur*. The data in Fig. 1 of J. M. Mitchison, A. Sveiczler & B. Novak (above) are difficult to analyse as the smoothing function (‘smooth’) seems to

have obliterated any observed variations such as those at 70–75 min and 90–95 min in the rate graph. In any case, the raw data of their Fig. 1 appear compatible with an exponential function and would show this if plotted using semi-logarithmic coordinates. The variability of the data (although no error bars are shown) blurs the distinction between bilinear or exponential growth.

Stephen Cooper

The ‘central question’ for Cooper must surely be whether there is a simple exponential growth law without RCPs for the whole cell cycle. This is clearly not so, since there are RCPs at the beginning and end of the constant length period. The third RCP during the growth period is conspicuous in *wee1* mutants (ignored by Cooper, though he has had the data), but less so in wild-type cells because the change in rate is smaller. In his supplementary correspondence, Cooper says that his Fig. 1 “definitely shows that length growth is exponential and not bilinear”. This is highly misleading, since Fig. 1 only shows

the exponential model and not the bilinear model, which would be extremely similar to an exponential on this semi-logarithmic plot. Indeed he finds that the numerical distinctions (r^2 values) between the different models are negligible. He has therefore to rely on the very weak argument that an exponential is preferable because it has fewer parameters and is simpler. This pre-judges the issue between simplistic models and the real life of cells. He ignores the fact that we have used a much more sensitive method which is equivalent to sequential differentiation in our Fig. 1, which shows a cell with a clear bilinear pattern. Smoothing processes have to be used with care since they obviously reduce variations but they are needed with ‘noisy’ data such as raw cell lengths. What Cooper does not mention is that our data used in his Fig. 1 had been smoothed by the same process used in our Fig. 1. Error bars, incidentally, are not appropriate in this work since individual cells grow at different rates and have different RCPs.

J. M. Mitchison, A. Sveiczler and B. Novak