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Size Control Models of *Saccharomyces cerevisiae* Cell Proliferation

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By using time-lapse photomicroscopy, the individual cycle times and sizes at bud emergence were measured for a population of *Saccharomyces cerevisiae* cells growing exponentially under balanced growth conditions in a specially constructed filming slide. There was extensive variability in both parameters for daughter and parent cells. The data on 162 pairs of siblings were analyzed for agreement with the predictions of the transition probability hypothesis and the critical-size hypothesis of yeast cell proliferation and also with a model incorporating both of these hypotheses in tandem. None of the models accounted for all of the experimental data, but two models did give good agreement to all of the data. The wobbly tandem model proposes that cells need to attain a critical size, which is very variable, enabling them to enter a start state from which they exit with first-order kinetics. The sloppy size control model suggests that cells have an increasing probability per unit time of traversing start as they increase in size, reaching a high plateau value which is less than one. Both models predict that the kinetics of entry into the cell division sequence will strongly depend on variability in birth size and thus will be quite different for daughters and parents of the asymmetrically dividing yeast cells. Mechanisms underlying these models are discussed.

The kinetics of proliferation of mammalian cells is well described by the transition probability model (2, 24, 26). In its original form (19, 32), the theory suggested that the cell cycle comprised a constant B phase (with variance) from a little before the initiation of DNA synthesis through cell birth and an A state, in the G1 period, from which cells escaped into the B phase with constant probability (*P*) per unit time. An important question is to see how far these concepts apply to free-living, single-celled eucaryotes.

One organism that has already received some attention is the budding yeast *Saccharomyces cerevisiae*. The key point of commitment in the yeast cell cycle is a point in the G1 period of the unbudded state called start (8). Traverse of start leads rapidly to initiation of DNA synthesis and bud emergence and ultimately, even in the absence of growth (12, 15), to cell division (10). The yeast cell cycle is asymmetrical, with daughter cycle times (D) being longer than parent cycle times (P) (9, 16). (Note that, because of the budding mode of growth, cells that are effectively siblings are traditionally referred to as parent, or mother, and daughter cells.) To account for these observations, a model of the cell cycle has been suggested (9) that proposes that budded cells divide asymmetrically into larger parents and smaller daughters and, to traverse start, a cell has to attain a critical cell size, V_c. At cell separation, parent cells will be $\geq V_c$ and can thus reenter the cycle immediately. Daughter cells at cell separation will be $< V_c$ and thus require a period of growth before traversing start; hence, $D > P$. Many of the quantitative predictions of this deterministic model have been verified (16, 35). Additional pieces of evidence for a size control over start have included the following observations. (i) Inhibition of growth leads to inhibition of cell number increase and not vice versa; proliferation is dependent upon growth and not the other way round (14). (ii) The time taken from birth of a cell (at cell separation) to bud emergence depends on birth size; small cells take longer to initiate a cycle (14). (iii) Starved cells accumulate as small cells before start (12, 14).

In contrast, it has been suggested that traverse of start is probabilistic (22, 23, 27-30), based on the following observations: (i) parent cells (born $\geq V_c$) should initiate a cycle immediately, yet the time to bud emergence is variable (14); (ii) cells accumulated at start by mating pheromone or by mutation, when released, show approximately first-order kinetics of entry into the cycle, rather than a synchronous round of initiation, which is found in cells released from an accumulation just

after start (28, 29); (iii) differences in proliferation rates can be expressed as differences in the exponential rate constant, k, for traverse of start ($P = 1 - e^{-k}$) (22, 32).

In principle, it is possible to reconcile these ideas, for example, by including a growth period in the transition probability model (17, 29, 35), a period applicable only to daughter cells, which they enter at birth and during which they grow to attain the minimum size necessary to enter the A state, which is equivalent to start. All cells then have a constant probability per unit time of leaving the A state. This two-component, tandem model (36) predicts that the distribution of cycle times of daughters will be caused by both an exponential term and variability in birth size.

To investigate these ideas further, I have made simultaneous measurements of the sizes and cycle times of individual cells undergoing balanced growth over two complete generations.

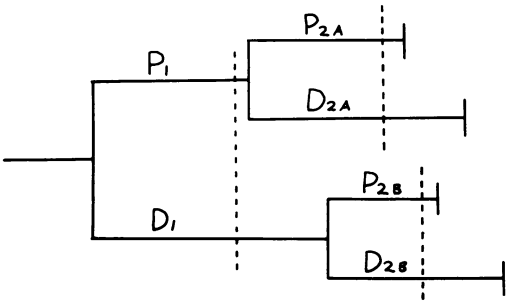

FIG. 1. Genealogical relationship of cells under study. The lines bifurcate at successive bud emergences. The cycle times of sibling daughter and parent (e.g., P_1 and D_1) were measured from successive bud emergences and thus are common for the budded period of the parent, cell division being approximately shown by the dashed lines.

MATERIALS AND METHODS

Yeast strain. *S. cerevisiae* haploid strain A364A (8) was used.

Exponential growth of yeast cells. Cells of strain A364A were grown in YEPDA liquid medium (1% yeast extract, 2% peptone [Difco], 2% glucose, 0.04% adenine) at 30°C to reach exponential growth. A sample of cells was then looped onto a flat block of YEPDA 5% agar medium in a filming slide, covered with a cover slip to produce a tight contact between agar and glass, sealed with wax to limit desiccation (yeast cells grow fermentatively on glucose at high concentration), and placed on a microscope stage in a 30°C temperature-controlled room. The cells were observed with phase-contrast optics with a long-working distance condenser at ×100 total magnification and filmed at 1-min intervals with commercial Kodachrome 25 film. Measurements of the timing of bud emergence, the genealogical relationships, and cell volume were taken from the processed film displayed on a screen. Timings were accurate to 1.6 min (95% confidence limits), and volumes, calculated from the major (a) and minor (b) axes of the cells and assuming $V = \pi ab^2/6$, were accurate to approximately ±10% (95% confidence limits).

RESULTS

Balanced exponential growth. To make quantitative comparisons between the predictions of the models, it is necessary to have populations of cells in balanced exponential growth (to avoid changes in the absolute values of any parameters) and to follow all cells for the same specified number of generations (to avoid growth bias and cutoff bias). The criteria used for establishing balanced growth were (i) exponential increase in number of cells under examination, (ii) parallel exponential increase in total cellular volume, and (iii) cycle times of first- and second-generation parents equal; cycle times of first- and second-generation daughters equal. Since it was difficult to monitor cell division (9), bud emergence was used as the temporal marker in the cycle for both scoring of doubling in cell number and the start or end of a daughter or parent cycle (Fig. 1). Although a slight lag in proliferation rate was seen during the first hour of filming, the data on exponential growth satisfy the three criteria for both population doubling time (Fig. 2) and individual cycle times (Table 1), except for second-generation daughters, which have shorter cycle times than first-generation daughters, just significantly different at the 5% level. Nevertheless, the calculated population doubling time from Table 1 was 84.1 min, using the mean values of D and P of the 324 cells and the age distribution of $e^{-\mu D} + e^{-\mu P} = 1$, where $\mu = \ln 2/\tau$ (16), identical to the measured values from Fig. 2.

Cycle time distributions. The distributions of cycle times as measured between successive bud emergences of daughters and parents are shown in Fig. 3, plotted as α plots (32), which are the percentages of unbudded cells, on a logarithmic scale against time. The parent α curve is close to a straight line after initial curvature but is better fitted by a shallow sigmoid curve. The daughter α curve is a pronounced sigmoid shape over a wider range of cycle times (52 to 198 min). A β plot (19, 24) of differences in sibling parent and daughter cycle times again shows a sigmoid curve with distinct asymmetry in cycle times, 94% of daughters having longer cycle times than their (sibling) parents. The three curves do not fit with a simple transition probability model (2, 32) as extended to budding yeast, since (i) the parent and daughter α curves do not become exponential after initial curvature; (ii) the poststart peri-

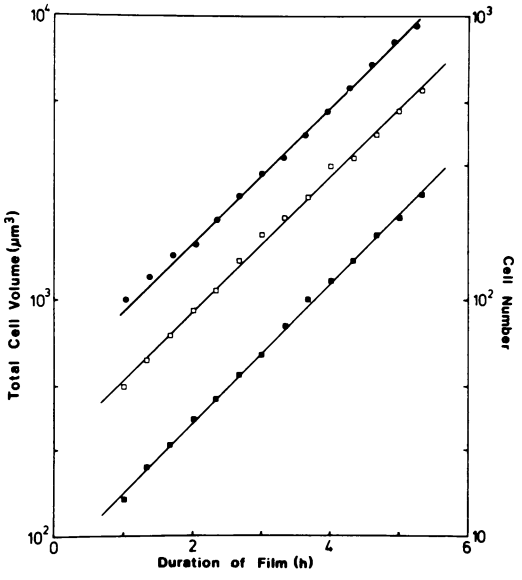

FIG. 2. Growth of a population of cells in a filming slide. The cells were inoculated onto a special slide (see text), and filming started 14 min after inoculation and continued for 6 h. Symbols: ●, total cell number of entire field (see Table 1); ■, total cell number of seven clones; □, total cell volume of the same seven clones. Doubling times were 84, 82, and 84 min, respectively.

od (equivalent to duration of the B phase, T_B) should be the same for parents and daughters since they are contiguous during the budded phase (Fig. 1), yet, if the initial curvature of the α curves is taken as evidence for variance in T_B (32), the mean value is 15 min longer in daughters; and (iii) if there were only an exponentially distributed component causing variability in sibling A state times and thus in cycle times, the β curve would eventually become a straight line whose intercept on the *x* axis when extrapolated back to 100% on the *y* axis would be 15 min (the difference in T_B). The shape of the β curve indicates an additional source of variance which, as shown by the α curves, must principally occur in daughter cells.

Cell volume at bud emergence. The volumes of cells at bud emergence were measured (Table 1), assuming yeast cells to be regular prolate spheroids. The volume of a parent cell at bud emergence will be strongly dependent upon its history. Parents get proportionately larger with genealogical age, values varying from a 7 to 23% increment per cell generation (11, 13). Older cells will separate at a large size and thus reinitiate at a larger size. Daughter cells, on the other hand, are smaller than the parents from which they separated (9). Since the distribution of birth sizes and bud emergence sizes overlaps only slightly and the two values for individual cells are only loosely correlated (17), size at bud emergence must be a function of the size at which daughters traversed start and not simply a function of birth size. The distribution (Fig. 4) shows considerable variability in size at bud emergence, the distribution being approximately normal with a coefficient of variation of 25%. This observation alone contradicts the predictions of the deterministic size control model since cells which are variable in size at birth should be similar in size at start (and thus bud emergence). Of the cells, 15% are even larger than the average size of their mothers at the next bud emergence.

DISCUSSION

Neither the simple transition probability model nor the simple deterministic model adequately accounts for the data on individual cells in steady-state culture shown in Fig. 3 and 4. The tandem model, in which cells attain a critical size before they enter the start state from which they exit with first-order kinetics, fares better since it successfully predicts (i) deviation from first-order kinetics in α curves for daughters, the magnitude depending on the birth size distribu-

TABLE 1. Cell cycle parameters of strain A364A[a]

Cell	Mean ± SD cycle time (min) of:		Mean ± SD vol at bud emergence (μm³)
	First-generation (P_1 and D_1)[b]	Second-generation ($P_{2A,B}$ and $D_{2A,B}$)[b]	
Parent	68.9 ± 11.0	70.0 ± 16.6	40.3 ± 13.9[c]
Daughter	104.1 ± 27.3[d]	94.6 ± 24.8[d]	29.7 ± 7.5

[a] The film described in the legend to Fig. 2 contained 51 clones; 11 clones were not analyzed because one had not grown, one had an extended lag period, and 9 became either out of focus or merged with other clones. The remaining 40 clones gave 111 parent and daughter cycle times for one generation. Seven clones were selected beforehand solely on the basis of clarity late in the film to give second-generation cycle times. The seven clones (Fig. 2) gave 51 measurable parent and daughter cycle times, only one daughter and one parent being out of focus.

[b] For P_1 and D_1, $n = 111$; for $P_{2A,B}$ and $D_{2A,B}$, $n = 51$.

[c] Includes parents of different genealogical ages.

[d] Significantly different at 5% level, using Student's *t* test and comparing D_1 with pooled D_{2A} and D_{2B} cells.

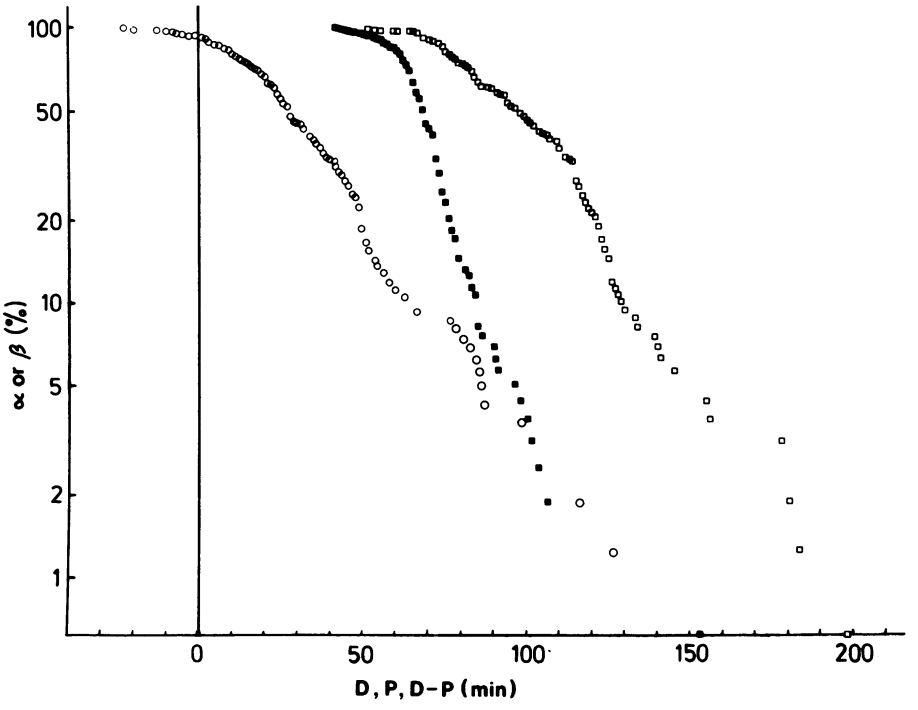

FIG. 3. α Plot and β plot of parent and daughter cycle times. The α plot is the percentage of 162 cells still to produce their next bud as a function of cycle time. The β plot is the percentage of 162 pairs of sibling cells with differences in cycle times $\geq t$. Note that siblings are P_n and D_n (see Fig. 1) and that $\Delta t = D_n - P_n$, rather than $|D_n - P_n|$ (20). Symbols: ■, α plot for parent cells; □, α plot for daughter cells; ○, β plot for sibling cells.

tion; (ii) deviation from exponential decay in the β curve for the same reason; and (iii) daughter cycle time being greater than parent cycle time because of the initial size requirement in daughters. However, the model cannot account for the nonexponential α curve for parent cells nor the exact shape of the distribution of sizes of daughters at bud emergence. Since yeast cells grow continuously through the cell cycle (5), the tandem model predicts that the distribution of daughter bud emergence sizes should be similarly approximately exponential when redrawn as percentage unbudded cells against volume and plotted on a logarithmic scale (γ curve). When the histogram distribution shown in Fig. 4 is redrawn in this fashion (Fig. 5), there is an approximately straight-line portion of the γ curve but also a substantial region of initial curvature. To account for this, the tandem model would have to be modified to include substantial variance in the critical size V_c, and this could also explain the nonexponential α curve.

A simple alternative to the wobbly tandem (WT) model can be constructed in which a single control point has both deterministic and probabilistic elements. Conventional deterministic models normally assume a step-function-like response when cells reach a particular critical size, the probability of initiating a cycle increasing rapidly from 0 to 1 (7). I suggest that there is indeed a size-monitoring system in cells which controls proliferation and which is sensed by the start mechanism in yeast but that the control is sloppy so that, as cells increase in size, the probability smoothly increases from zero to some high value, probably less than 1. After initiation, there follows the cell division sequence, a fixed period (with small variance) leading to division.

Data which are in agreement with this sloppy size control (SSC) model can be derived from Fig. 4. It is possible to calculate the hazard rate, which is the conditional probability per unit time of an unbudded daughter cell of a given size producing a bud before it reaches the next size interval (Fig. 6). No daughter cells bud below 10 μm^3; then, as the size increases, there is an increasing probability per unit time that a cell will bud, rising to a high (possibly plateau) level of approximately 0.6 per 16 min (which is the unit time; see legend to Fig. 6). The standard errors get very large when only a few cells are involved, but the overall shape of the curve is quite clear and consistent with the model suggested above. In calculating the data shown in Fig. 6, it was assumed that unbudded cells grew

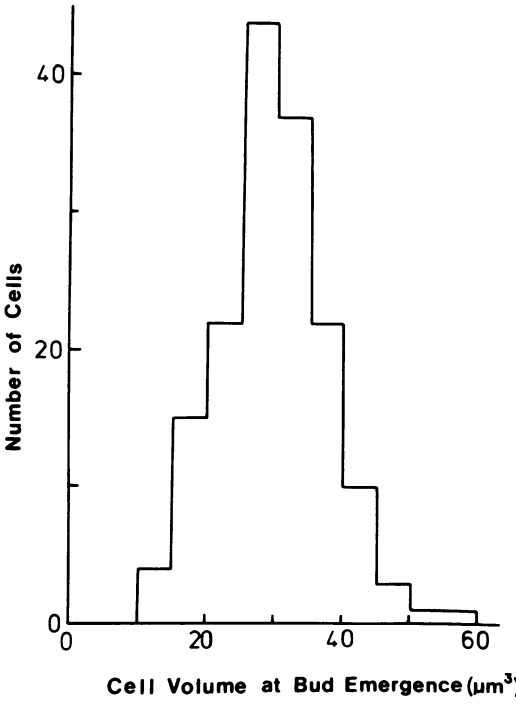

FIG. 4. Distribution of volumes of daughter cells at bud emergence. The distribution is of 159 cells and consists of the 162 referred to in Table 1 (D_1, D_{2A}, D_{2B}), except for 3 cells whose volume could not be accurately measured. The volumes refer to the number of cells budding in the appropriate 5-μm^3 volume interval.

in volume in a linear fashion (1), although good data on this point are lacking. If volume increase is exponential, then the numerical values change, but the shape of the curve remains unaltered, and the standard errors overlap for the two curves (data not shown). The existence of a small amount of overlap between the distributions of birth sizes and bud emergence sizes of daughters means that the calculated hazard rates for values of ≤ 22.5 μm^3 are less than their true values, since not all daughters in the population are free to initiate a bud at that size.

The observed kinetics of cell proliferation will thus strongly depend on birth size. Very small cells (<20 μm^3; daughters only) will not bud until they have grown, showing size control over cycle time; average-sized cells (20 to 40 μm^3; daughters and parents) will show an increasing probability of budding the larger they are born. The control, however, will be loose, and there will be considerable heterogeneity both in size at bud emergence and cycle time; large cells (>40 μm^3; parents only) will be born in the flat portion of the curve, and thus no size control will be seen, only an exponential distribution of cycle times, since the probability of budding is remaining the same even as the cells grow. Traverse of start can thus assume either determined or random properties, depending upon the experimental protocol and the size of the cells.

A correct hazard rate curve cannot be drawn for mothers because they are born large. However, it is possible to estimate average probability solely from the kinetics of cell proliferation and average growth rate. From the quasi-straight-line portion of the parent α curve shown in Fig. 3, a P value of 0.74 per 16 min is obtained, together with an average volume at bud emergence of 40.3 μm^3 (Table 1). The calculated point fits reasonably well in the plateau portion of the curve (Fig. 6). Both the WT and SSC models qualitatively account for the nonexponential α curves (Fig. 3). Heterogeneity in parent birth size implies that some cells are born into the rising part of the hazard rate curve (SSC model) or in the variable V_c region (WT model), which in both cases would indicate a reduced rate of traverse of start producing the flatter portion in the tail of the α curve. Similarly, a very variable birth size for daughters (17) implies variable probability (SSC model) or dif-

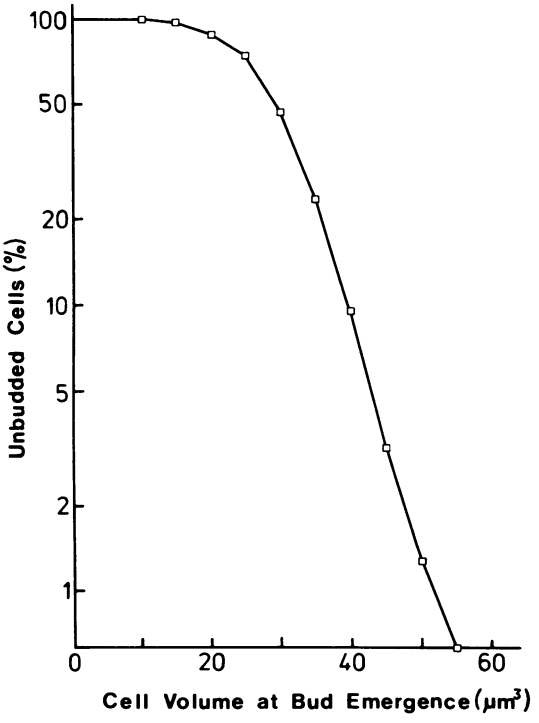

FIG. 5. γ Plot for daughter cells. The data are taken from Fig. 4 and replotted as percentage of unbudded cells (as a fraction of the whole population) plotted on a logarithmic scale against cell size.

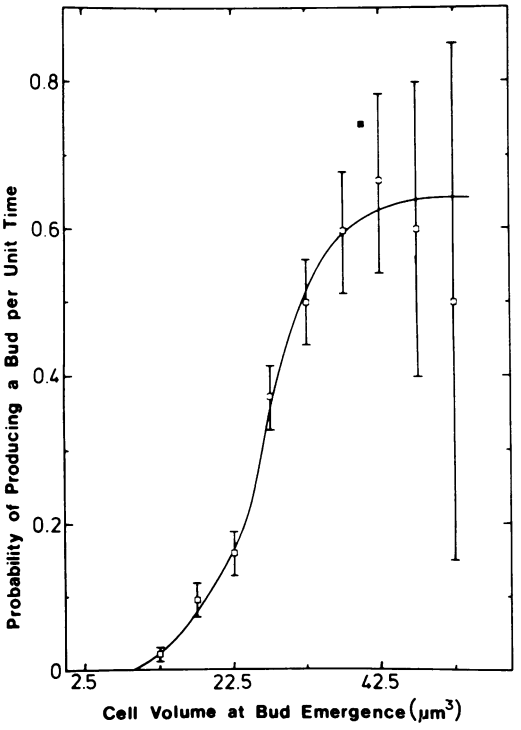

FIG. 6. Conditional probability per unit time of a daughter cell of given volume initiating budding (hazard rate). The values were taken from Fig. 4, and P was calculated as $P = (N_v - N_{v+1})/N_v$, where N_v is the number of unbudded cells at volume interval V, and N_{v+1} is the number of unbudded cells remaining at the next volume interval. The standard errors (bars) were calculated as standard error = $\{P(1 - P)/n\}^{1/2}$, where n is the number of unbudded cells arriving at that size interval. The average cell volume calculated from Fig. 2 was 24.0 μm^3, but this calculation is based on cells dividing into two at bud emergence and therefore underestimated cell volume in proportion to the budded period (B). This was estimated to be 50 min (Fig. 3 and reference 16); therefore, the true average cell volume was 24 × 2 exp (B/τ) = 36.2 μm^3. Each cell therefore grows, on the average, 26.2/τ = 0.31 μm^3/min, and therefore takes 5/0.31 = 16 min to grow a 5-μm^3 size interval (Fig. 6). ■, Average P for parents.

ferent times to traverse the variable V_c (WT model), producing α curves with pronounced curvature. Both models also account for random release from a start block and synchronous release from a poststart block (28, 29). A start block does not inhibit growth, except with *cdc*-25 (14, 21), so cells will continue to the plateau portion of the hazard rate curve (SSC model) or accumulate in the A state (WT model) and will thus show first-order kinetics on release. Cells blocked after start will probabilistically traverse the start event and after release will show the cumulative probability of bud emergence, i.e., synchrony.

Since it is possible to arrest by starvation larger parents of older genealogical ages (14) and modulate initiation size rapidly without concomitant growth (18), it is probably not volume itself which is monitored by the cell but some cellular component which is produced as a constant fraction of macromolecular synthesis (enabling growth to be measured) and turned over at a fast rate so that the current physiological status of the cell is surveyed rather than its previous history (7, 18). By studying exponentially growing cells under balanced growth conditions when all cellular parameters are, on the average, increasing in parallel and the cell is in some kind of steady state, cell volume is probably as good a measure of cell size as is possible.

Both the WT and SSC models have been simulated by computer modeling (P. E. Green and A. E. Wheals, manuscript in preparation). Interestingly, both require seven parameters to be specified for a precise description of the models, five of which are common and numerically equal. Both models provide good fits to the data and are experimentally indistinguishable in this protocol. Nevertheless, the models imply completely different methods and mechanisms of the control of cell proliferation.

For example, one of the few schemes that can explain regulation of size, even in cytoplasmic amputation experiments and in asymmetrical division of yeast, is the unstable inhibitor dilution model (7). There is circumstantial evidence from *Schizosaccharomyces pombe* for an activator as well as an inhibitor (6) and evidence in *S. cerevisiae* implicating the spindle pole body (SPB) and its related microtubule organizing center in start (3, 20). Furthermore, duplication of the SPB is the first known event poststart (3). Figure 5 is also reminiscent of a velocity-substrate concentration plot of an enzyme showing a heterotropic allosteric response with positive cooperativity. Putting these points together, a simple mechanistic scheme to explain the SSC model could be as follows: (i) a component of the SPB-microtubule organizing center initiates the cycle by allowing duplication of the SPB; (ii) the component is kept under repression by an unstable inhibitor which acts as an allosteric effector reversibly binding to and inactivating the SPB component at high concentration and existing as the unbound form at low concentration, thus freeing the SPB from repression; (iii) the inhibitor is kept at a constant amount directly proportional to gene dosage, its concentration thus falling twofold during the cycle (7); and (iv) chance fluctuations in the interaction between a single SPB component and a few molecules of inhibitor produce the stochastic differences between otherwise identical cells and the probabilistic traverse of start. This scheme has some

similarities to those proposed (on quite different grounds) for both *S. pombe* (6) and *S. cerevisiae* (30).

On the assumptions of the SSC model, we can see from Fig. 6 that there is a change in probability value of from 10 to 90% of the maximum over an approximately 2.5-fold change in cellular volumes (and thus a change in postulated effector concentration), which implies a Hill coefficient of 4.8. This suggests a small number of components (perhaps four) and is well within the range of known allosteric interactions. Since the true probability curve is flatter (17; see above), the value becomes closer to the estimate of 2.4 (30) which has been obtained from quite different experiments.

It is also possible to produce a plausible mechanism in terms of the WT model. The model has similarities to the two-transition model devised for mammalian cells (2), except the first transition is normally rather than exponentially distributed. The mechanism for the second transition could be as described previously (33) for the transition probability hypothesis. The mechanism for the first transition could be accurate monitoring of a critical size by a titration mechanism (7) which is, however, normally distributed between cells, or imprecise monitoring of a critical size or imprecise specifying of a critical size, as has been suggested previously (34) for bacteria. Experimental distinctions between these possibilities will await knowledge of the molecules involved.

Independently of its exact distribution, all appropriate studies have found considerable cycle time variability (9, 17, 28, 29), and this seems to be located in the unbudded, prestart, G1 period (17, 28, 29). The importance of this observation is that it suggests that this period is dispensible and exists solely so that homeostatic regulation of size can occur, but whose presence or absence is not crucial to the welfare of the cell (4, 31). In this sense, the G1 period before start is of a quite different nature to the cell division sequence after start, all of whose events are necessary for normal growth and division.

Although the transition probability model postulates no size control component to account for the kinetics of cell proliferation (2), the ideas in this paper are relevant to its evaluation. If mammalian cells have a similar SSC system, for example, and are normally born in the plateau part of the hazard rate curve, purely random kinetics will be seen. Alternatively, small cells born into the slope of the hazard rate curve will show size control. Similarly, if the WT model is correct, then size will be an important determinant only in cells that are born small and are well below the V_c; larger cells will show transition probability kinetics. One experiment to investigate size did indeed show a size-related component in determining cell proliferation kinetics for smaller cells (25). The discrepancy between probabilistic and deterministic models may be more apparent than real, since the view expressed here is that they may be simply two aspects of the same mechanism (20).

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