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Profiles of Random Change During Aging Contain Hidden Information about Longevity and the Aging Process

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Many different morphological and physiological changes occur during the yeast replicative lifespan. It has been proposed that change is a cause rather than an effect of aging. It is difficult to ascribe causality to processes that manifest themselves at the level of the entire organism, because of their global nature. Although causal connections can be established for processes that occur at the molecular level, their exact contributions are obscured, because they are immersed in a highly interactive network of processes. A top–down approach that can isolate crucial features of aging processes for further study may be a productive avenue. We have mathematically depicted the complicated and random changes that occur in cellular spatial organization during the lifespan of individual yeast cells. We call them budding profiles. This has allowed us to demonstrate that budding profiles are a highly individual characteristic, and that they are correlated with an individual cell's longevity. Additional information can be extracted from our model, indicating that random budding is associated with longevity. This expectation was confirmed, providing new avenues for exploring causal factors in yeast aging. The methodology described here can be readily applied to other aspects of aging in yeast and in higher organisms.

Introduction

Aging is a complicated process. It is characterized by a multitude of seemingly unrelated phenotypes, to which a causal role is difficult to ascribe. Although the net effect is a progressive decline in functional capacity, advancing age is heterogeneous in its manifestations both within and between individuals of a species, and it is not easily described as a linear process. This nonlinearity can be seen as aging proceeds in fits and starts at various levels of biological organization, from the molecular to the organismal. Unlike development, it is difficult to discern an unfolding pathway or program during aging. The apparent random nature of age changes perhaps derives from the features of the aging process described above. This stochasticity constitutes a challenge to the understanding of aging. The highly interactive attribute of biological systems and their emergent properties suggest that a top–down description of aging may be a useful first step, which can inform subsequent attempts to identify its critical components. One such approach postulates that change is a cause and not simply an effect of aging (Jazwinski *et al.*, 1998).

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Individual yeast (*Saccharomyces cerevisiae*) cells divide a limited number of times (Mortimer & Johnston, 1959). The mean number of divisions available to a yeast cohort is characteristic for a given yeast strain; in other words, it is genetically determined. It is the number of divisions and not calendar time that is the measure of the yeast replicative life span (Müller *et al*., 1980). Although individual yeasts are mortal, the population is immortal and is not subject to clonal senescence, which occurs with individual cells from metazoans. Yeast mortality rate increases exponentially as a function of age (Pohley, 1987; Jazwinski *et al*., 1989), but it displays a deceleration at older ages (Jazwinski *et al*., 1998). Furthermore, yeasts undergo a panoply of age changes (Jazwinski, 1999a), many of which represent functional decline. Thus, yeasts are prone to an aging process, by the usual definition. Many of the broad physiological principles may be the same for aging in yeast and in higher organisms (Jazwinski, 1996).

Some 20 genes have been shown to play a role in determining yeast life span. These genes describe a wide array of different biochemical functions (Jazwinski, 2000), suggesting that there is more than one pathway or process that is important for yeast longevity. Indeed, several mechanisms of aging have been discerned. Some of these are metabolic (Kirchman *et al*., 1999; Ashrafi *et al*., 2000; Jiang *et al*., 2000; Lin *et al*., 2000). Another is resistance to environmental insults, including thermal (Shama *et al*., 1998a,b), oxidative (Wawryn *et al*., 1999; Barker *et al*., 1999), and ultraviolet radiation (Kale & Jazwinski, 1996) stress. Genetic instability (Sinclair & Guarente, 1998) and gene dysregulation (Jazwinski, 1996; Kim *et al*., 1996; Smeal *et al*., 1996; Kim *et al*., 1999a; Imai *et al*., 2000) may be yet others.

Budding pattern in yeast is an expression of cellular spatial order (Drubin & Nelson, 1996). Haploid yeasts produce daughter cells in an axial budding pattern, such that consecutive buds are formed at close proximity to the previous site of budding. The potential relevance of this manifestation of cell polarity to aging was proposed earlier (Jazwinski, 1993). Viewed at the population level, there is decay in this spatial order as a function of replicative age, and there are genetic manipulations that concomitantly modulate budding pattern and life span, suggesting a link between the two (Jazwinski *et al*., 1998). The increase in random budding of a yeast cohort as it ages may, however, simply be a reflection of a tendency for yeasts to bud randomly as they get older and/or just before they die. At the individual level, cellular budding pattern profiles during the lifespan exhibit apparent random changes (Jazwinski, 1999b). In the present study, we mathematically describe budding pattern profiles of individual yeast cells. We demonstrate that these profiles are correlated with the longevity of individual cells and that they provide detailed information concerning features of spatial order that may play a causal role, providing avenues for experimental verification.

Experimental Procedures

STRAINS AND GROWTH CONDITIONS

S. cerevisiae SP1-1 (*MAT**a***, *leu*2, *ura*3, *trp*1, *ade*8, *can*1, *his*3, *gal*2) was used (Jazwinski *et al*., 1998). The yeast cells were cultured on synthetic SD medium containing the required nutritional supplements and 2% galactose as carbon source at 30°C, as described before (Sun *et al*., 1994). The solid medium contained 2% agar.

LIFESPAN DETERMINATION AND BUDDING PATTERN ANALYSIS

Lifespans and budding patterns were determined, as before (Jazwinski *et al*., 1998). The analyses were performed on individual cells growing on plates containing solid medium. Newborn cells were deposited at isolated spots to initiate the determinations with the aid of a micromanipulator and observed microscopically (Kim *et al*., 1999b). Each bud produced by these cells was removed and discarded, after allowing the next consecutive buds to appear to allow the determination of budding pattern. The total number of buds produced by the cell was its lifespan in generations. Axial budding was defined by the appearance of consecutive buds in close proximity on the same hemisphere of the cell. Random budding was defined by the appearance of consecutive buds on the opposite hemispheres of the cell. No polar budding, as defined by the appearance of consecutive buds on the opposite poles of the cell, was observed in this study. These definitions are operational with no assumptions made regarding underlying mechanisms. The budding pattern of the first cell division was not available, by definition. After the final bud was produced, the majority of the cells lysed, although some cells lingered but lost refractility. The budding profiles of short- and long-lived cells, as defined in the Results, can be obtained from the corresponding author.

STATISTICAL ANALYSES

Data were entered into Microsoft Excel 2000 spreadsheets. Means, medians, and standard deviations were determined, and ANOVA was performed using the resident Excel statistical package. Mann–Whitney statistics were applied to survival data, Pearson correlation analysis was carried out, and discriminant functions were generated using StatMost 3.00 (DataMost Corp.). The significance of differences between mean discriminant function scores was ascertained from the F-distribution, using Hotelling's T^2 (Harris, 1975).

Results

BUDDING PATTERN PROFILES OF INDIVIDUAL YEAST CELLS DURING AGING

The lifespans and budding pattern profiles during these lifespans of 151 individual yeast cells were determined (Experiment I). The survival curve of this aging cohort is shown in Fig. 1. Cells with lifespans shorter than 5 generations were excluded from further analysis. These cells were considered to have died prematurely. Five generations is only about 25% of the cohort mean lifespan. These cells constituted only 6% of the aging cohort. In addition, cells in which any budding cycle could not be classified as axial or random were discarded from the analysis. This problem occurs when a bud detaches from a cell before the next bud has appeared. This difficulty was rarely encountered in this study, because cells were examined for buds relatively frequently. Only 5% of the cells were thus eliminated. The mean, median, and maximum lifespans of the remaining cells from the aging cohort were 19, 18, and 34 generations, respectively (Table 1). These cells were analysed further.

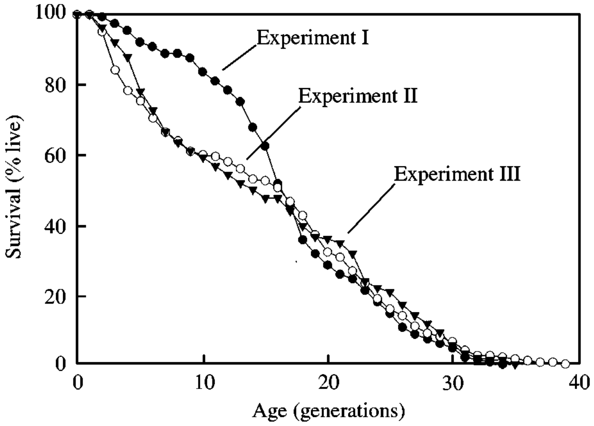

FIG. 1. Survival analysis of the cohorts of aging yeast cells used in the study. Experiment I (151 cells), Experiment II (200 cells), and Experiment III (163 cells).

The cells in Experiment I showed alternating axial and random budding patterns that resulted in profiles of change during aging that appeared stochastic and unique for each cell. Six randomly chosen examples of these profiles are depicted in

TABLE 1

Characteristics of the aging yeast cell cohorts used in the study

Experiment	Cells analysed				Cells discarded (number)	
	Total number	*Lifespan (generations)*			Unclassified*	Early death†
		Mean	Median	Maximum		
I	135	18.71	18	34	7	9
II	145	19.1	19	39	2	55
III	127	18.56	19	35	1	36

*At least one budding cycle that could not be classified as axial or random.
†Total lifespan less than 5 generations.

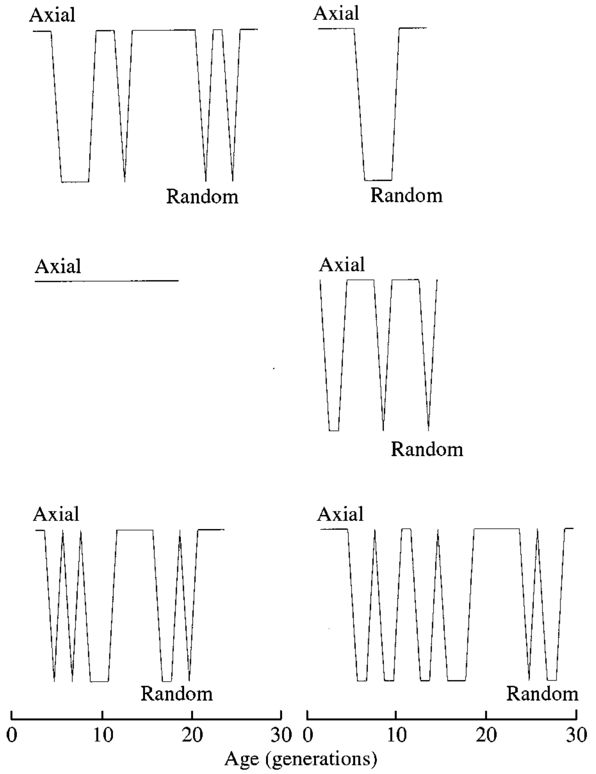

FIG. 2. Budding profiles of a sample of six cells used in the study. Note that plots do not begin at the origin, because buddings can only be classified starting with the second one.

Fig. 2. We wanted to know whether such budding profiles were in any way informative about the longevity of the cells to which they belonged. In other words, is cellular spatial organization (polarity) correlated with lifespan?

A MODEL RELATING BUDDING PROFILE TO LONGEVITY

To examine whether a relationship exists between the budding profile and longevity of individual cells, we needed to reduce the complicated features of these profiles to variables that could be readily quantified. In other words, we wanted to describe and analyse the patterns depicted by these budding profiles as fully as possible, rather than making any *a priori* assumptions regarding their individual features. We therefore chose several characteristic features of these profiles that together provide an extensive portrayal of their total geometry. These features (variables) included: (1) the total number of axial buddings (x_1), (2) the total number of random buddings (x_2), (3) the number of changes in budding pattern from axial to random and from random to axial (x_3), (4) the longest stretch or run of axial buddings expressed as the number of buddings in this stretch (x_4), (5) the longest stretch or run of random buddings expressed as the number of buddings in this stretch (x_5), (6) the mean length of all of the stretches of axial buddings (x_6), and (7) the mean length of all of the stretches of random buddings (x_7). The variables x_1–x_7 were used to construct a linear equation:

$$B = a_1x_1 + a_2x_2 + a_3x_3 + a_4x_4 + a_5x_5 + a_6x_6 + a_7x_7, \qquad (1)$$

in which a_1–a_7 are coefficients that assign an appropriate weight to each of the variables and B is a derived variable that we call the bud print. Equation (1) defines points in seven-dimensional space. In essence, it reduces this space to a single dimension that is encapsulated in the value of B, which can be readily manipulated further, even though it is more difficult to conceptualize. B is formally equivalent to a discriminant function in multivariate statistics, and it is operationally treated as such here. A linear equation is appropriate because we describe features of the budding profile, and there is no reason to postulate interactions between features of a profile as such.

Next, it was necessary to choose values for the coefficients a_1–a_7 that would enhance the relationship of the entire ensemble of predictor variables to the longevity of individual cells (criterion variable); that is, we derived the discriminant function. We used this approach, because of the complicated nature of the budding profile that could be at the heart of the relationship we were testing. An additional virtue of this approach is that it allows the data to speak for themselves, pointing out those elements of cell polarity that are most important for generating a relationship between budding profile and longevity. This, in turn, facilitates the formulation of new hypotheses for testing.

The strategy, described above, for selection of the coefficients requires their mutual optimization so that the equation that is the outcome most effectively relates B to longevity. This is a mathematical maximization process. We have found that the discriminant function module in the StatMost program is suitable for this task, although discriminant analysis routines provided in other commonly available statistical packages are up to the task. The application of this routine requires the grouping of the individual cells on the basis of the factor according to which we wish to distinguish among them, in this case longevity. We chose two groups for this purpose. The first was short-lived and defined as cells whose lifespan was less than the population median. The second was long-lived and defined as cells whose lifespan was greater or equal to the population median. The choice of discrete groups is apt because survival is not distributed normally (Fig. 1). Formally in discriminant analysis, the procedure is to find the linear combination of predictor variables which maximizes between to within association by selection of an optimal row vector of coefficients (a'), providing for maximum separation of the criterion groups (Harris, 1975).

The procedure outlined above was applied to the data of Experiment I, in which median cell lifespan was 18 generations. This resulted in a model relating budding profile to longevity written as

$$B = -0.1018x_1 - 0.2995x_2 - 0.1155x_3 - 0.2119x_4 - 0.0064x_5 - 0.0091x_6 + 0.1286x_7. \quad (2)$$

Equation (2) assigns a value of B to each individual cell that is related to the cell's longevity. B had a value of -1.12 to -8.33 (Fig. 3(a)). However, a clear dichotomy was seen. B for short- and long-lived cells described distinct intervals that displayed little overlap (|0.186|). Only 5 out of a total of 135 cells were present in this interval. The mean B for the short- and long-lived cells were -3.30 and -5.91, respectively. This difference is significant ($p \ll 0.001$). Thus, the degree of differentiation between these two groups of cells is beyond that expected by chance alone. A cut-off value of B (-4.4) was selected that reduced the number of cells (3) that were incorrectly classified as short- or long-lived to a minimum.

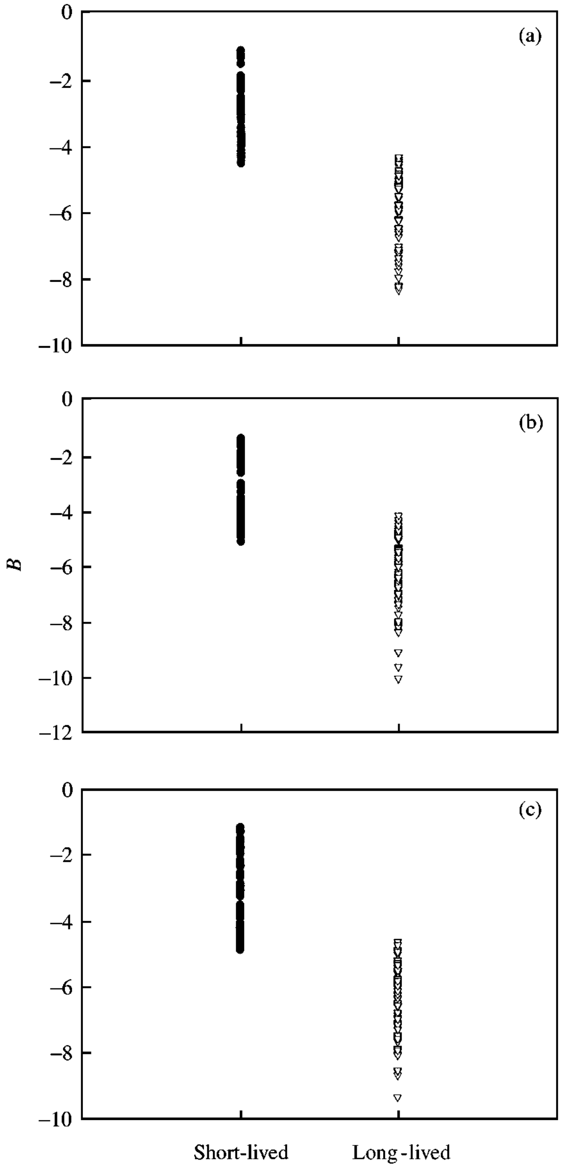

FIG. 3. B values of short- and long-lived cells. B values were calculated for cells using eqn (2). They are plotted for cells grouped as short-lived (< median lifespan) and long-lived (⩾ median lifespan). (a) Experiment I (18 generations median lifespan). (b) Experiment II (19 generations median lifespan). (c) Experiment III (19 generations median lifespan).

To provide a view of the effect of a more complete description of the budding profile on the accuracy of the classification of the cells, we examined the overlaps between the B value intervals for the short- and long-lived groups and the number of cells in these overlaps when the number of variables (x_1–x_7) was altered (Fig. 4). This was carried out by generating equations of the

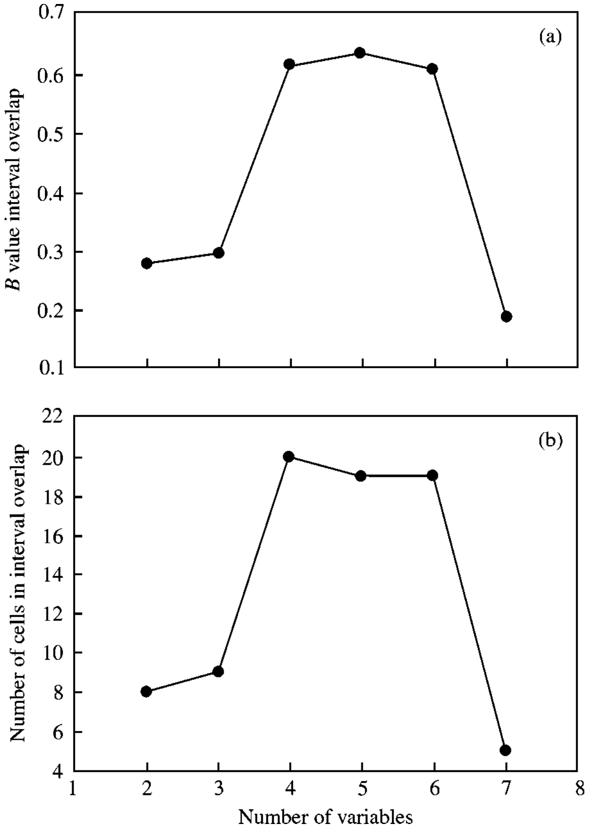

FIG. 4. Effect of the number of budding profile variables on discrimination between short- and long-lived cells. (a) Equations of the form of eqn (1) were constructed, using variables x_1 and x_2 alone (2 variables), x_1, x_2, and x_3 (3 variables), and so forth with the remaining variables x_4–x_7, in sequence. The coefficients (a_i) were derived as for eqn (2), and the B values for each equation were calculated for all of the cells in Experiment I. The absolute value of the overlap between the B value intervals for short- and long-lived cells is plotted for each of the equations. (b) The number of cells in the overlaps of the B value intervals for short- and long-lived cells is plotted as a function of the number of variables used to generate the equations in (a).

same form as eqn (2), using variables x_1 and x_2 and then sequentially adding variables x_3–x_7, one at a time, to create new equations. The size of the overlap at first increased with the inclusion of additional variables (Fig. 4(a)). However, it ultimately reached its lowest size when all seven variables were included. The number of cells included in the overlaps displayed similar behavior, attaining its nadir when all seven variables were taken into account (Fig. 4(b)).

Equation (2) allows us to ascertain whether the budding profiles of individual cells are unique, as an examination of their graphic representations suggests (Fig. 2). Among all of the cells examined (Experiments I, II, and III), the B values were identical for 20 pairs (40 cells total). This constitutes just 10% of the total number of cells analysed. It is plausible that the inclusion of additional features of the budding profiles would reduce this number further. Consistent with this notion, there were only 2 pairs (4 cells total) that were long-lived that had identical budding profiles to each other.

UTILITY OF THE MODEL AS A PREDICTOR OF LONGEVITY

Even though the number of cells in Experiment I, which were used to derive eqn (2), was large in comparison with the number of predictor variables, this equation could be the result of chance associations, which is a fundamental problem in multivariate procedures such as discriminant analysis (Harris, 1975). The observed accuracy of prediction on the sample on which the discriminant function was developed is always spuriously high, because this function capitalizes on chance relationships in that sample, as a matter of course. To rule this out, it was necessary to validate the utility of eqn (2) for categorizing cells as short- or long-lived on the basis of an independent sample of cells. In fact, we carried out this exercise in two separate experiments (Experiments II and III). Some of the salient characteristics of the two samples analysed are summarized in Table 1 and Fig. 1. The lifespans of the cells in Experiments I–III did not differ significantly, as determined by both the Mann-Whitney test ($p > 0.05$) and ANOVA ($p > 0.08$). It is evident, however, that underlying these similar population parameters is a richness of budding profiles, which is evidenced by their uniqueness. This renders the further testing of eqn (2) described here all the more important.

The predictor variables x_1–x_7 were tabulated for each of the cells in Experiments II and III. These were inserted into eqn (2) to generate the characteristic B value for each cell. The distribution of the B values, sorted by short- and long-lived cells, is shown in Fig. 3(b) and (c) for Experiments II and III, respectively. It can be seen that the mean, the range and the overlap interval of these B scores were similar to those observed in

TABLE 2
Validation of the discriminant function relating budding profile to longevity

Experiment	Predicted	Actual		Total
		Short-lived	Long-lived	
I	Short-lived	61	2*	63
	Long-lived	1*	71	72
	Total	62	73	135
II	Short-lived	55	4*	59
	Long-lived	9*	77	86
	Total	64	81	145
III	Short-lived	52	3*	55
	Long-lived	1*	71	72
	Total	53	74	127

*Misclassified cells.

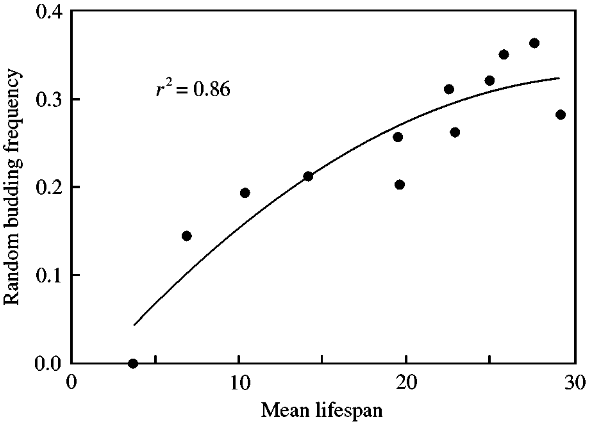

FIG. 5. Longer life span is associated with a higher frequency of random budding. Cells from Experiment III were grouped according to their frequency of random budding. This is the ratio of the number of random buddings divided by the total number of buddings for each individual cell. The mean lifespan of these grouped cells is plotted. The line is the second order polynomial regression. r^2 is 0.86. (A linear regression resulted in $r^2 = 0.82$.) Similar results were obtained for the cells in Experiments I and II.

Experiment I. The cells were then classified as either short- or long-lived on the basis of the previously established cut-off score of -4.4, as defined earlier. The misclassification rate into the two groups was low in all three experiments ($< 10\%$) as seen in Table 2.

The predictive value of eqn (2) evidenced in Table 2 indicates that this equation accurately relates the budding profiles of individual cells to their longevity. We conclude that the budding profiles of yeast cells are correlated with their individual lifespans. Although this association does not establish the underlying causal factors on its own, it can point to candidates for such factors.

INTERPRETATION OF THE MODEL

Since eqn (2) is valid for additional samples of aging cells, it establishes a correlation between a cell's bud print and its longevity. Further analysis of this equation can indicate the relative importance of features of the bud print in this association. Such features expressed as the predictor variables are strong candidates for major causal factors in lifespan determination. One avenue for the identification of relevant variables is the analysis of the standardized coefficients of eqn (2). The absolute values of the standardized coefficients were 0.0897, 0.2805, 0.0662, 0.1335, 0.00002, 0.00012, and 0.00166 for coefficients a_1–a_7, respectively. It is evident from this tabulation that variable x_2 (number of random buddings) has the largest impact on B (in the context of all the other variables), at least two-fold larger than any of the others. These results led us to predict that the number of random buddings is a key factor associated with longevity.

We further explored the potential of the number of random buddings (x_2) as a candidate causal factor in lifespan determination. This was done by analysing the mean of the lifespan of cells grouped according to frequency of random budding. When cells from Experiment III, as an example, were examined, it was evident that longer lifespan was associated with higher frequencies (Fig. 5). Since frequencies, a standardized value, rather than absolute numbers were used in this analysis, it was not biased according to lifespan. This analysis provides support for a critical role of random budding in determining yeast lifespan.

Discussion

We have reduced the complicated profiles of budding pattern changes exhibited by aging yeast cells to a simple linear equation [eqn (2)]. This facilitated the examination of the relationship of these profiles to the longevity of individual yeast cells. Our data indicate that these profiles are correlated with longevity. Indeed, the equation very accurately indicates whether the cell is short- or long-lived. By closer analysis of the coefficients in eqn (2), we predicted that the number of random buddings has a strong association with the longevity of an individual cell. This prediction was indeed verified. Greater frequencies (normalized to a cell's lifespan) of random budding were associated with longer lifespans. The reduction of the budding profile to eqn (2) also allowed us to confirm that the budding profiles are highly individual to each yeast cell.

The association of random budding with longer life was unexpected. At a population level, we had previously found that yeasts bud randomly at a higher frequency as they age (Jazwinski *et al*., 1998). This was also the case in the experiments described in this report. Axial buddings seem to be preferred early in the lifespans of most cells (data not shown). Thus, there must be a point in the lifespan of an individual cell at which the appearance of random buddings is a predictor of long life. Perhaps, yeast cells have as long a lifespan as they do because they develop a greater propensity to bud randomly. However, this issue remains open for future studies.

Yeast replicative lifespan is measured by the number of buddings, which also enter into the description of the budding profile. Indeed, $x_1 + x_2 + 1$ is a precise measure of lifespan, which is the reason for the relatively small B value interval overlap encountered when the variables x_1 and x_2 or x_1, x_2 and x_3 were used (Fig. 4(a)). These relationships are trivial. However, eqn (2) provides more than a simple tabulation of the number of buds a cell produces. It provides a picture of the way in which the budding pattern changes. This additional information content decreases the overlap between the groups of short- and long-lived cells and reduces the number of misclassified cells. Further to the point, our purpose here was to determine whether the budding profile of an individual cell, which is characterized by apparently stochastic polarity changes and is unique to individual cells, is informative of that cell's longevity. The precipitous decline in B value overlap interval and the associated decrease in the number of cells in this interval (Fig. 4) when a more complete description of the budding profile is used suggest that, in fact, this is what has been accomplished. In discriminant functions, the importance of particular predictor variables can only be measured in the context of the entire constellation of these variables. The non-trivial nature of eqn (2) is evidenced by the fact that it places great weight on the number of random buddings, which constitute at most about one-third of the total buddings during the lifespan of a yeast cell. Several other models that place weight on various features of the budding profile have been compared to eqn (2) (Appendix A). None have as good a predictive value.

Equation (2) can be modified to contain additional variables. Although its predictive value is very high, this might reduce the misclassification rate even further, and it might assign a totally unique B value to every cell. This equation describes well the "shapes" contained within the budding profile, but it does not specify the order of their appearance during the lifespan. This may prove a fruitful avenue for further work, because the place of random buddings during the lifespan appears to be associated with longevity, as discussed above.

The model described by eqn (2) predicts the importance of random budding for longevity. This directs our attention to this aspect of cellular spatial order as a target for further hypothesis generation. In fact, hypotheses can be formulated and tested even at the molecular level. Such experimentation can provide evidence for causal effects.

The association of random budding with longevity is consistent with the qualitative model of aging developed earlier (Jazwinski *et al*., 1998). It is the number of random buddings not the length of the stretches of random budding that appears to be important. Random budding is a departure from the norm of axial budding in haploid yeast. The intensification of this budding pattern, which is associated with aging at the population level, is related to a longer lifespan of individual cells. This is reminiscent of the epigenetic stratification of aging yeast populations into cells whose mortality rate continuously increases and those for whom it plateaus (Jazwinski *et al*., 1998). It is the intensification of aging processes that leads to the stratification described by the qualitative model of aging mentioned above. It remains to be determined how and when in the lifespan the machinery of bud site selection may impinge on longevity. It is important to note that under other experimental conditions, eqn (2) may be replaced by another equation of the same form without affecting the association of budding profile with longevity. However, the relative importance of the predictor variables may change, providing valuable information about the aging process.

The methodology highlighted in our study may be applicable to other parameters of aging organisms. For example, the gene dysregulation (Jazwinski, 1996) that occurs in yeast, and in other organisms, may contain information about the aging process that can be extracted using procedures similar to those described in this study.

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APPENDIX A

Alternative Models of Budding Profiles

Other models relating budding profiles to longevity have been explored. These included mathematical maximizations based on discriminant functions of the form of eqn (1). Out of a total of

5040 (7!) possibilities combining the seven predictor variables, equations for three different sets of variables are presented.

$$B_1 = -0.2201x_1 - 0.3899x_2 + 0.0578x_3 + 0.3884x_7, \quad \text{(A.1)}$$

$$B_2 = -0.1799x_1 - 0.3025x_2 - 0.0559x_3 - 0.0832x_5 - 0.1316x_6 + 0.2532x_7, \quad \text{(A.2)}$$

$$B_3 = -0.4379x_2. \quad \text{(A.3)}$$

These equations were chosen for the following reasons. Equation (A.2) drops x_4, which seems to be a variable that decreases the accuracy of the discriminant function combining variables x_1, x_2, and x_3 (Fig. 4). Equation (A.1) adds variable x_7, which has a dramatic effect on the accuracy of eqn (2), to variables x_1, x_2, and x_3. Equation (A.3) examines the accuracy of x_2 alone as a predictor, because the number of random buddings is closely associated with longevity according to eqn (2). The *B* value overlap intervals and the number of cells in these intervals are tabulated below for comparison with Fig. 4.

	Absolute value of overlap interval	Number of cells in overlap interval
B_1	0.13	7
B_2	0.12	10
B_3	3.50	116

Of these three equations, only eqn (A.1) appears to approach the accuracy of eqn (2). However, the standardized coefficients for eqn (A.1) are 0.434, 0.492, 0.017, and 0.016 for a_1, a_2, a_3, and a_7, respectively. This indicates that the number of axial (x_1) and random (x_2) buddings have approximately the same relative importance for longevity. It is clear from Fig. 5 that an association exists between random budding and lifespan. Similarly, we searched for a correlation between the frequency of axial budding of individual cells and lifespan, using the Pearson test. The correlation coefficient was -0.403 ($p = 0.322$), indicating lack of an association. On this basis, we reject eqn (A.1) as a useful model relating budding profile to longevity. It is worth reiterating that our goal was not to create a parsimonious model based on features of budding profiles assumed to be important, but to generate a derived variable (*B* value) that encompasses most or all of the features of the budding profile in their native complexity.

Additonally, two other expressions which contained no coefficients but only the variables were written, to compare with the discriminant analysis approach we have used. These expressions were

$$B_4 = (x_1 - x_2)/(x_1 + x_2), \quad \text{(A.4)}$$

$$B_5 = x_1/x_2. \quad \text{(A.5)}$$

The *B* value intervals for short- and long-lived cells ranged from -0.07 to 1.0 and -0.11 to 1.0, respectively, for B_4, and from 0.7 to 7.0 and 0.8 to 8.0, respectively, for B_5. This constituted a nearly complete overlap. Virtually all the cells fell into these overlap intervals.

The definitions of short-lived, long-lived, and of the variables used in this Appendix are the same as in the body of this article.