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Relationship between the Fraction of Cells of Different Genealogical Ages and their Cycle Times in *Saccharomyces cerevisiae*: A Theoretical Analysis

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In *Saccharomyces cerevisiae*, two separate subpopulations can be distinguished visually: parents and daughters, the former receiving the major portion of the septal material as a bud scar. These scars persist as stable structures, and so can serve as indicators of the number of times a cell has undergone division—its genealogical age. Daughters can be similarly classified according to the generation of the parent cell from which they separated. The actual fraction of each class of cells depends on their lifetime. Previous attempts to derive a relationship between these two entities were restricted to the special case in which all parent cells possess a common cycle time, regardless of their genealogical age, and so do all daughters. Experimental evidence suggests that such extreme assumptions are unwarranted; they are also unnecessary. The relationship between the number of parent cells in any particular genealogical age class is shown here to depend solely on their lifetime and on those of younger parent cells. The expressions obtained consist exclusively of closed elementary algebraic forms, and the calculation of age at division from cell fraction is easy and straightforward. The analysis is then extended to cover the case of heterogeneous daughter cells. The results there turn out to be simpler still: the fraction of daughter cells of a particular genealogical age is dependent on its own doubling time only, and not on those of younger cells.

Introduction

Cells of the budding yeast *Saccharomyces cerevisiae* form a chitin-containing ring around the site of their emerging bud. The septum that is synthesized at this location prior to separation splits asymmetrically at cell division. Because of this, two separate subpopulations can be distinguished visually: parents and daughters, the former receiving the major portion of the septal material as a bud scar. These scars persist as stable structures, and so can serve as indicators of the number of times a cell has undergone division, its genealogical age. The parent population can thus be subdivided into first-generation parents, second-generation parents, and so on.

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Daughters can be similarly classified according to the generation of the parent cell from which they separated (Beran *et al*., 1969; Egilmez & Jazwinski, 1989), although current staining procedures are not able to provide persistent markers and the faint ring structure formed by the so-called birth scar disappears within a cycle or two.

The actual fraction of each class of cells depends on their lifetime. Previous attempts (Hartwell & Unger, 1977; Lord & Wheals, 1980) to derive a relationship between these two entities were restricted to the special case in which all parent cells possess a common cycle time, regardless of their genealogical age, and so do all daughters. Experimental evidence suggests that such extreme assumptions are unwarranted (Hartwell & Unger, 1977; Egilmez & Jazwinski, 1989); fortunately, they are also unnecessary. We show below that the relationship between the number of parent cells in any

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particular genealogical age class depends only on their lifetime and on those of younger parent cells. The relationship in the case of daughter cells turns out to be even simpler.

The reason that cell cycle time varies with genealogical age, increasing slowly at first and then ever more rapidly, is not at all clear, although there are indications that an accumulating deficiency in energy metabolism may be involved (Jazwinski, 1993). But whatever the mechanism, empirically the cycle time of an individual cell has been found to be an accurate predictor of when it is destined to die and, as such, constitutes a valuable biomarker for the aging process since it reflects physiological or functional age rather than nominal age, unlike the genealogy of the cell or its size (Jazwinski, 1990). The purpose of the present communication is to make this property readily accessible to the experimental mycologist.

Theory

Consider a population of *S. cerevisiae* in balanced exponential growth. Let $n_g(a)$ be the number of cells at age a with g scars (that is, of genealogical age g), and let τ_g be the age at which these cells divide. In principle, in order for a steady state to exist, there must be a maximum value of g, call it G, such that cells with G scars continue to grow slowly if at all but are no longer able to divide; in practice, loss of reproductive capacity occurs at $G=25$ or so (Johnston, 1966; Jazwinski, 1990).

Since every parent cell with g scars gives rise at division to one newborn parent cell with $g+1$ scars and one daughter cell with $g=0$ scars,

$$n_{g+1}(0)=n_g(\tau_g), \quad \text{for } g=0, 1, \ldots, G, \tag{1}$$

and

$$n_0(0)=\Sigma n_g(\tau_g), \tag{2}$$

where the summation extends over all g from 0 to G.

The culture is considered to be in balanced exponential growth, and so each component increases exponentially with the same doubling time τ. The fraction F_g of cells of genealogical age g is $\int n_g(a)\,\mathrm{d}a$, so that

$$F_g=\int n_g(0)2^{-a/\tau}\,\mathrm{d}a=\alpha^{-1}n_g(0)[1-2^{-\tau_g/\tau}], \tag{3}$$

where $\alpha\equiv\ln(2)/\tau$ is the growth rate. This provides an explicit expression for τ_g,

$$\tau_g=-\alpha^{-1}\ln(1-\alpha F_g/n_g(0)), \tag{4}$$

which, together with the recurrence relationship for $n_g(0)$,

$$n_g(0)=n_{g-1}(\tau_{g-1})=n_{g-1}(0)2^{-\tau_{g-1}/\tau}, \tag{5}$$

allows us to compute any τ_g from a knowledge of $F_0, F_1, \ldots, F_g$ and α:

(i) τ_0 is computed from F_0 by using eqn (4) with $g=0$. (It will be shown below that $n_0(0)$ is equal to α.)
(ii) $n_1(0)$ is determined from eqn (5) by setting $g=1$ and using the τ_0 from the preceding step.
(iii) τ_1 is then obtained from F_1 by using eqn (4) with $g=1$ and $n_1(0)$ from the preceding step.
(iv) The process is repeated for successive values of g as far as the experimental resolution permits.

It remains to show that $n_0(0)$ is indeed equal to α.

The total number of cells in the culture is given by ΣF_g. From eqn (3),

$$\begin{aligned}\Sigma F_g&=\alpha^{-1}\Sigma n_g(0)[1-2^{-\tau_g/\tau}]\\&=\alpha^{-1}\Sigma n_g(0)-\alpha^{-1}\Sigma n_g(\tau_g)\\&=\alpha^{-1}\Sigma n_g(0)-\alpha^{-1}\Sigma n_{g+1}(0)\end{aligned}$$

from eqn (1). Since all summations are over g from 0 to G, the intermediate terms in this last expression cancel, leaving just $\alpha^{-1}n_0(0)-\alpha^{-1}n_{G+1}(0)$. But cells of genealogy G do not divide, so that $n_{G+1}(0)$ is zero and the total number of cells is simply $\alpha^{-1}n_0(0)$. In order to interpret the F_g as frequencies rather than numbers, one merely sets the total number of cells to unity, so that finally

$$n_0(0)=\alpha. \tag{6}$$

By substituting for $n_g(\tau_g)$ in eqn (2), it is easy to show that, not surprisingly, the total frequency of newborn cells is equal to 2α, the same as in symmetrical binary fission (Powell, 1956).

Heterogeneous Daughter Cells

Up to this point we have treated daughter cells (those with $g=0$) as a homogeneous group. It would appear, however, that this may not be justified (Beran *et al*., 1969; Egilmez & Jazwinski, 1989). We therefore generalize our analysis to cover this situation also. The only effect a heterogeneous daughter population has on the expressions derived above for the parent cells, is to require a reinterpretation of τ_0. For the moment, it is sufficient to define it formally as the solution to the equation $n_1(0)=\alpha 2^{-\tau_0/\tau}$. A more biological interpretation is presented below.

Let $m_g(a)$ be the frequency of daughter cells of age a separated from parents with g scars, and let θ_g be their

doubling time. Since a parent cell with g scars gives rise on division to one newborn parent cell with $g+1$ scars and one daughter cell of genealogical age g, and since that is the only source of these newborn cells, then

$$m_g(0) = n_{g+1}(0) = n_g(\tau_g). \tag{7}$$

Whenever a daughter cell divides, it gives rise to one newborn parent cell with a single scar and one daughter cell of genealogical age 0. Since this obtains for all dividing daughter cells, regardless of genealogical age,

$$m_0(0) = n_1(0) = \Sigma m_g(\theta_g). \tag{8}$$

But $n_1(0) = n_0(\tau_0) = n_0(0)2^{-\tau_0/\tau}$, and so it follows from eqn (8) that

$$n_0(0)2^{-\tau_0/\tau} = \Sigma m_g(\theta_g) = \Sigma m_g(0)2^{-\theta_g/\tau},$$

or

$$2^{-\tau_0/\tau} = [\Sigma m_g(0)2^{-\theta_g/\tau}] \div [\Sigma m_g(0)], \tag{9}$$

where we have used the identity

$$\Sigma m_g(0) \equiv n_0(0), \tag{10}$$

the total frequency of newborn daughter cells.

Equation (9) provides us with the biological interpretation for τ_0: as expected, it is the average doubling time of the daughter cells, suitably weighted according to the size of the fraction. This can be recast as

$$2^{-\tau_0/\tau} = \langle 2^{-\theta_g/\tau} \rangle, \tag{11}$$

where the angle brackets denote averaging according to eqn (9).

Let G_g represent the fraction of daughter cells of genealogical age g. Then

$$\begin{aligned} G_g &= \int m_g(0)2^{-a/\tau}\, \mathrm{d}a \\ &= \alpha^{-1} m_g(0)[1 - 2^{-\theta_g/\tau}] \\ &= \alpha^{-1} n_{g+1}(0)[1 - 2^{-\theta_g/\tau}] \end{aligned} \tag{12}$$

from eqn (7). This allows us to obtain θ_g from experimental values of G_g since the $n_{g+1}(0)$ are known from the parent distribution derived above. Note that, unlike in the case of the parents, G_g depends only on θ_g itself and not on the doubling times of daughters of lesser genealogical age.

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