Experimental Gerontology, Vol. 33, No. 6, pp. 571–580, 1998

0531-5565/98 $19.00 + .00

PIIS0531-5565(98)00029-1

EPIGENETIC STRATIFICATION: THE ROLE OF INDIVIDUAL CHANGE IN THE BIOLOGICAL AGING PROCESS

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Abstract—Aging is a complex process. It consists of a diverse assortment of seemingly random manifestations that occur in the individual, the mutual relationship and impact on mortality of which is frequently obscure. We derive a simple equation to model the aging process based on scale invariant and increasing change. The solution to this equation indicates that this change itself, irrespective of its quality, is the cause and not simply the effect of aging. This model establishes loss of homeostasis as a fundamental feature of aging. The model is deterministic, but it supports the stochastic nature of age changes. Paradoxically, this model states that a sufficient augmentation of aging processes results in a lack of aging. Experimental evidence in support of this model is presented that spans the levels of population mortality rates, cellular spatial organization, and gene dysregulation. *© 1998 Elsevier Science Inc.*

Key words: aging, longevity, mortality rate, cell polarity, silencing, nonlinear systems

INTRODUCTION

AGING IS a complicated process that is characterized by a decline in functional capacity (Finch, 1990). This functional capacity appears to encompass a disparate array of physiological parameters, including metabolic capacity, the efficiency of stress responses, integrity of gene regulation, and genetic stability (Jazwinski, 1996). The orderly unfolding of developmental programs that result in the generation of complex structures and associated functions contrasts starkly with aging, which manifests itself in a heterogeneous manner, even in genetically homogeneous populations maintained under uniform conditions. This heterogeneity is evident both at the level of the functional decrements accompanying aging and in the longevity of individuals in an aging cohort that ultimately derives from these decrements.

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(*Received* 23 *February* 1998; *Accepted* 7 *May* 1998)

MATERIALS AND METHODS

Strains and growth conditions

The *Saccharomyces cerevisiae* strain YPK9 (*MAT***a**, *ade*2-101^{ochre}, *his*3-Δ200, *leu*2-Δ1, *lys*2-801^{amber}, *trp*1-Δ63, *ura*3-52), derived from the diploid YPH501 (Sikorski and Hieter, 1989), was used in the mortality studies. *S. cerevisiae* SP1-1 (*MAT***a**, *leu*2, *ura*3, *trp*1, *ade*8, *can*1, *his*3, *gal*2), derived from SP1 (Sun *et al*., 1994), was used in the cell polarity studies. The *RAS*1 and *RAS*2 genes were deleted as described (Sun *et al*., 1994) in *S. cerevisiae* UCC519 (*MAT***a**, *ade*2, *his*3, *leu*2, *lys*2, *trp*1, *ura*3-52, *ppr*1) (Renauld *et al*., 1993), in which the *URA*3 gene is integrated at the right telomere of chromosome V, for the silencing studies. Yeasts were grown as described before (Sun *et al*., 1994) either on rich YPD medium or synthetic media containing the appropriate nutrients. When needed, 5-fluoroorotic acid was present at 1 mg/mL. *RAS*2 overexpression was obtained as before (Sun *et al*., 1994) in medium containing 4% galactose.

Life span determinations

Life spans were determined microscopically for individual cells by counting the number of buds produced and removing them by micromanipulation (Egilmez and Jazwinski, 1989). The determinations were initiated on newly born daughters. Prior to life-span determination cells were grown on medium containing glycerol instead of glucose as carbon source to eliminate any petites, lacking fully functional mitochondria from the analysis. Age is expressed as the number of generations or cell divisions completed. Mortality rate is the fraction of live cells that die during an interval (Jazwinski *et al*., 1989). The mortality rate studies were carried out with several cohorts of cells. The mean life spans and variances were calculated, and ANOVA was performed to determine whether individual cohorts could be combined. Mortality rates were calculated using the computer program MORTAL 1.0 designed and provided by James W. Curtsinger (jwcurt@vx.cis.umn.edu) at the University of Minnesota, and smoothed over a six-generation window by an algorithm contained in the program.

Budding pattern analysis

Budding patterns were determined by examining individual cells throughout their life spans. Budded cells were allowed to form a nascent bud to determine the relative orientation of two consecutive buds. If the new bud was adjacent to the previous one, the pattern was scored as axial. If it was located in the opposite hemisphere of the cell, it was classified as random. The results are expressed as the percent of the total live cells that budded randomly at any given age.

Statistical analysis

Means, standard deviations, and ANOVA on the mortality data, and standard errors and the *t*-test on the silencing data were obtained using the statistical package contained in Microsoft Excel version 7.0. Age-specific mortality data were analyzed by fitting different mortality models and maximum likelihood test (Fukui *et al*., 1993). The program Modest version 2.0 designed by Scott Pletcher (plet0005@tc.umn.edu) was used. The model fitting was performed by Scott Pletcher in J.W. Curtsinger's laboratory. The regression analysis of the budding pattern data was performed using SAS System for Windows release 6.11 by William Johnson in the Department of Biometry and Genetics at this institution (W. Johnson, C.-Y. Lai, and S.M. Jazwinski, in preparation).

RESULTS AND DISCUSSION

We describe or model organismal aging by postulating only one essential and universal property of this process, increasing change. The change referred to here is not a temporary physiological response followed by return to a baseline, such as the increase in heart rate during exercise. Temporary changes of this sort operate within the bounds of homeostasis. The aging change we postulate leads in consequence to alterations in homeostasis, as will be seen. Development also involves change, as alluded to above. Development is, however, programmed, even though it is endowed with some plasticity. Aging lacks this constraint, as it is not subject directly to natural selection (Rose, 1991). The changes that constitute aging occur with a certain probability at any point during the life span. They increase, and individually they need not be irreversible. The probability of change, in one direction or the other, can be expressed as e^{-x}, where e is the base of the natural logarithm and $x \geqslant 0$. An exponential expression is appropriate because the propensity to age is held to be multiplicative rather than additive. In fact, survival is distributed approximately log-normally as a function of age. The exponential equation is commonly used to describe the probability of change in physical systems.

We introduce the factor A to account for the effect of aging on this probability. This factor fulfills the postulate of increasing change. We describe the dynamics of this aging system by a difference equation (Drazin, 1992), because we are dealing with a series of discrete states. Thus, we have:

$$P_{n+1} = Ae^{-P_n} \quad \text{for } n = 0, 1, 2, 3 \ldots \tag{1}$$

in which x is replaced by P, a parameter that describes the state of the system and is related to the probability of change. P is a dimensionless variable that describes the propensity to age on a relative scale, with P of 0 equivalent to none. A large P indicates intense aging in approach to death. The variable n denotes consecutive states of P. $A \geqslant 0$. When $A = 0$, $P = 0$, and there is no aging. The difference between consecutive states of the system (Δ) described by equation 1 need not be small; that is, lim Δ need not be 0. This, and the lack of a requirement that the change be continuous and unidirectional, renders a difference equation appropriate. Equation 1 qualitatively models an aging system based solely on increasing change.

The solution of equation 1 is an iterative process for any given A, in which the result obtained, P_{n+1}, is inserted into the exponent in the next iteration ($n = 0, 1, 2, 3 \ldots$). This feature of the difference equation is apt because the aging system is endowed with "memory." The propensity to age is dependent on the previous state of the system and not on an arbitrary value of P. After a suitable number of iterations, the solution of equation 1 produces a constant value (stable fixed point) for P that can be plotted as a function of A to generate a bifurcation diagram (Fig. 1). This diagram exhibits an increase in the value of P that is asymptotic to 1 with A increasing from 0. However, a dramatic change occurs when A is between 2 and 3. A distinct bifurcation is seen with two constant solutions (stable two-cycle) for equation 1. One of these solutions consistently increases in value such that P approaches A. The other solution converges on 0 asymptotically. This behavior is characteristic of a nonlinear system (Drazin, 1992).

The iteration that is involved in the solution to equation 1 results in changing values of P that converge on the stable fixed point prior to the bifurcation in Fig. 1. This reflects the tendency of the aging system to ultimately reach an equilibrium, despite initial fluctuations in state imposed by change. Similar considerations apply to the iteration that results in convergence on the two fixed points of the two-cycle, past the bifurcation point in Fig. 1. In this case, the system

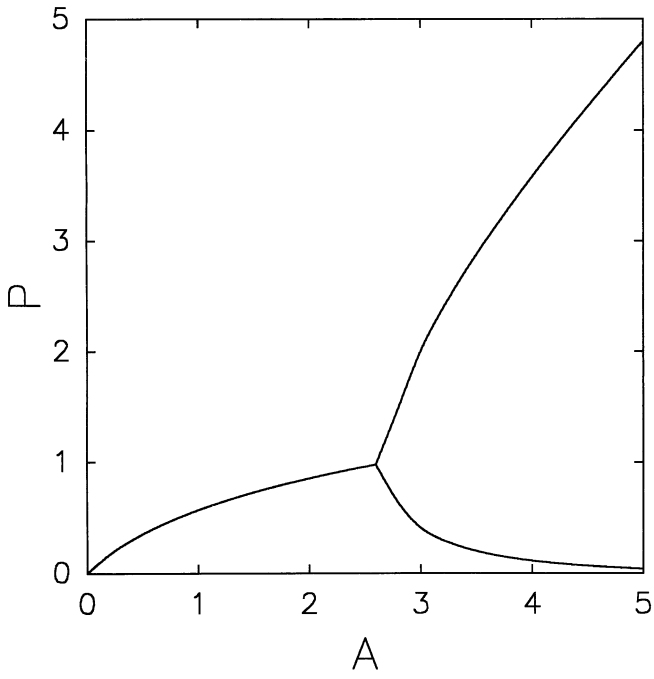

FIG. 1. Bifurcation diagram for equation 1. The solutions obtained by iteration in the interval $0 \leqslant A \leqslant 5$ are plotted. P_0 was set at 0 to denote the status quo. Numerical solutions to equation 1 were calculated by iteration using a simple program written in QBASIC by Roger L. West in this laboratory.

alternates between two states—one characterized by a high propensity to age (*P* converging on *A*), and the other by a low one (*P* converging on 0). Equation 1 is not solved in time but in consecutive states of the system (*n*). The value of *n* has no upper limit. The consequence of this is that the state of the individual aging system is indeterminate, and it can be represented by one or the other of the fixed points of the two-cycle.

It is noteworthy that factor *A* need not be large for the bifurcation in Fig. 1 to occur. Modest changes of two- to threefold, equivalent to the value of *A* at which the bifurcation appears, are often seen in various parameters during aging, such as in the expression of specific genes (Egilmez *et al.*, 1989). The solution of equation 1 does not depend on the initial state, P_0. Furthermore, it is not sensitive to small changes of initial states. Consistent with the later, the solution does not become chaotic.

Equation 1 portrays an aging system, in the first instance the intact organism. It can be used as easily to describe the behavior of components of this system at increasingly finer scale or level of biological organization from cellular to molecular. Equation 1 is generally applicable because we perceive that aging is fundamentally self-similar (scale invariant), though not identical, at different levels of organization. It is, therefore, fractal, at each level related to others by appropriate scaling functions. In the framework of our model, this means that equation 1 describes the dynamics of aging, and it permits the determination of the state of the aging system (*P*), at any scale (level), molecular, cellular, or organismal, as well as those intermediate. Many properties of organisms, including life span, scale as quarter powers of body mass. This has been

shown to result from their fractal nature (West *et al*., 1997). Equation 1 implies counterintuitively that, given a sufficient impact of aging (*A*) on any biological process, two stable outcomes (*P*) are the result of aging processes, at whatever the biological level that is analyzed. These outcomes are the tendency to maintain organization or homeostasis overall despite change and the loss of this organization, which dispose to survival or to death, respectively.

We decided to test the model of equation 1 using budding yeast. An individual yeast cell divides a limited number of times. Each time a daughter is produced that has the potential for a full life span. The probability that a yeast continues through this replicative life span decreases exponentially as a function of the number of divisions completed or age (in generations) (Pohley, 1987; Jazwinski *et al*., 1989). Yeasts undergo a variety of morphological and physiological changes, some decremental, as they proceed through their life spans (Jazwinski, 1993). Therefore, they age.

Is there evidence to support our model? The survival characteristics of a population are derived from the individual aging organism. Equation 1 predicts that an aging yeast cohort should become stratified epigenetically into a group that suffers from continuously increasing mortality rate and into one that ages slowly or not at all. This stratification could only occur when changes in the state of the system (*n*) are sparse. This is likely to happen at older ages. The generation time, or the time elapsed between consecutive cell divisions, increases exponentially with age in yeast (Egilmez and Jazwinski, 1989). If changes in the state of this aging system (*n*) are linked in some way to the completion of consecutive cell divisions or are proportional to the passage of generation time, these changes (*n*) will become rare, even almost nonexistent, as yeasts age. The mortality rate, which is the rate at which yeasts die at any given age, increases exponentially as a function of age (Pohley, 1987; Jazwinski *et al*., 1989). However, the mortality rate appeared to plateau for individuals surviving to older ages when a large enough aging cohort was examined (Fig. 2). The majority of the population suffered from an increase in the rate of aging during the life span, but a small minority appeared not to age at later ages. The first group is readily described by the increasing value of *P* in Fig. 1 following the bifurcation, which indicates the inevitability of death. The latter group is depicted by the decreasing value of *P* in Fig. 1 following the bifurcation, which indicates the potential for the lack of aging. It is interesting to note that the behavior of the population described here requires a certain minimum value of *A* in equation 1. If there is no increase with age of the changes that this equation describes or if it is below a certain value, no bifurcation takes place. Furthermore, there is no epigenetic stratification of the population, with the emergence of a nonaging group.

The plateau in mortality rate observed here with yeast has been described previously in medflies (Carey *et al*., 1992), *Drosophila* (Curtsinger *et al*., 1992), and *Caenorhabditis elegans* (Vaupel *et al*., 1994). It has not been readily explained by genetic or environmental factors. The epigenetic stratification (Jazwinski, 1996) described here provides such an explanation. The results with these organisms provide further support for the theoretical construct of equation 1.

Change is an intrinsic feature of an aging system (equation 1). Genetic and environmental factors can only affect such a system through factor *A*. Consequently, genetic and environmental effects should modulate the loss of organization or homeostasis that equation 1 describes. Such modulation would be detected in individual yeast cells. Other than mortality itself, one of the most global expressions of homeostasis that is measurable is at the biological level of spatial order. Equation 1 predicts that spatial order would be lost progressively during aging and that this aging change could be modulated by genetic manipulation.

An expression of spatial order in yeast is budding pattern. Haploid yeasts bud axially at a high frequency; that is, consecutive buds are located in close proximity to the previous site of

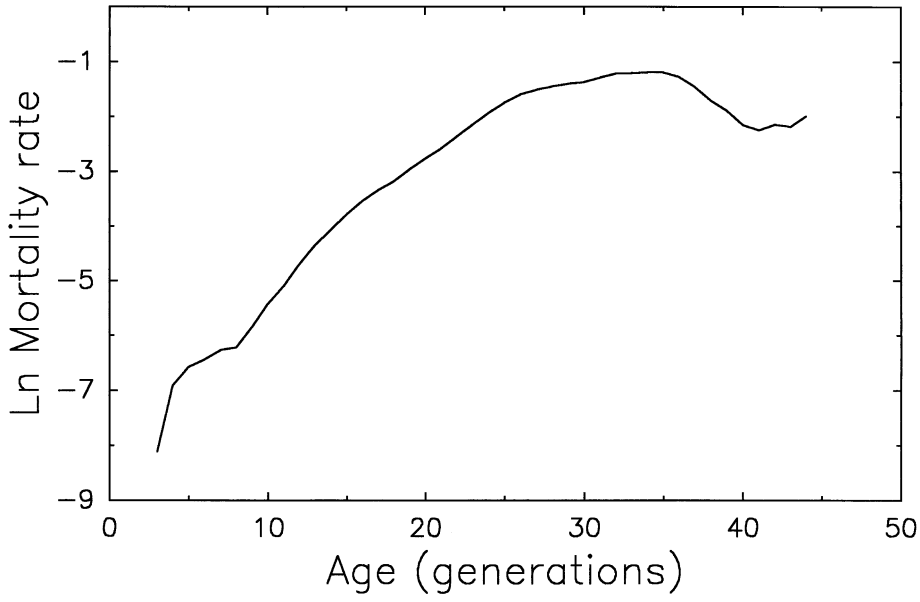

FIG. 2. Survival of yeast populations. Mortality rate (ln) is plotted as a function of age [$n = 508$, mean life span = 23 generations (SD = 6.02)]. A logistic model in which there is an exponentially increasing mortality at early ages and a plateau at older ages fit the data best, according to a maximum likelihood test ($p < 0.00001$).

budding (Drubin and Nelson, 1996). With age, there was a progressive decline in this manifestation of spatial order (Fig. 3). Older yeasts budded axially less frequently than younger yeasts. These results indicate a progressive loss of homeostasis or dysregulation at the cellular level. Nevertheless, a substantial fraction of the cell divisions remained axial even late in the life span, suggesting the existence of stability in spatial order alongside the obvious dysregulation in cell polarity. It is noteworthy that individual cells did not permanently change budding pattern at various points during their life spans. Instead, periods of random and axial budding of various duration were interspersed. Figure 4 shows the distribution of the frequency with which individual cells changed budding pattern during their life spans. These data demonstrate that the state of the system undergoes multiple reciprocal changes during aging, as predicted by equation 1. They also provide empirical support for the iteration inherent in the solution to equation 1. It will, however, be necessary to increase the number of observations to analyze the patterns of cell polarity change for any regularities.

Overexpression of the *RAS*2 gene postponed the decline in the frequency of axial budding during the life span (Fig. 3). The loss of spatial order was no longer monotonic, but instead, displayed a singularity evidenced by the breakpoint in the regression. In addition, overexpression of *RAS*2 reduced the scatter (residual variance) observed in the increase in frequency of nonaxial budding fourfold. This increased homogeneity provides further support for the enhancement in spatial order. These results suggest that *RAS*2 acts through factor *A*. *RAS*2 is a gene that plays a role in determining yeast longevity (Sun *et al*., 1994).

The significance of spatial order in aging is supported by the decrease in life span obtained by deletion of the *BUD*1 gene, which is involved in establishing cell polarity (data not shown).

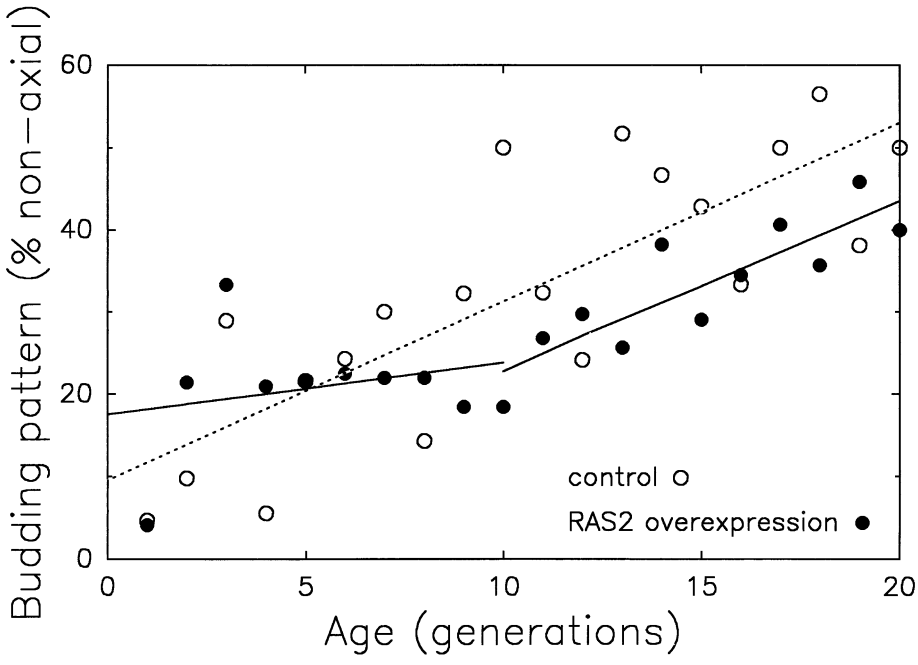

FIG. 3. Budding pattern during the yeast life span. The number of cells displaying a random (nonaxial) budding pattern expressed as percent of the live cells is plotted as a function of age. Only 1 out of almost 1,600 buddings scored was clearly polar, as determined by the emergence of a bud on the pole opposite the previous bud. The lines show linear regressions for cells overexpressing *RAS*2 (solid lines) ($n = 50$; $r^2 = 0.97$; slope a = 0.63, $p = 0.435$ [slope not different from 0]; slope b = 2.07, $p = 0.0025$) and for the control cells (broken line) carrying the empty vector ($n = 48$; $r^2 = 0.65$; slope = 2.17, $p < 0.0001$). There is no significant difference between slope b (2.07) on *RAS*2 overexpression and the slope (2.17) of the control ($t = 0.08$, $p > 0.50$). The residual variance in the postponed increase in nonaxial budding on *RAS*2 overexpression is significantly reduced compared to the control (23.8 vs. 93.6, $F = 3.93$, $p < 0.05$).

It is interesting to note that the *tea*1 gene in *Schizosaccharomyces pombe* is important in generating global spatial order (Mata and Nurse, 1997). The tea1 protein contains repeats belonging to the kelch family, which are present in cytoskeleton binding proteins. One of the homologues of tea1 is encoded by the *C. elegans spe-26* gene (Varkey *et al*., 1995), which plays a role in determining nematode life span (Van Voorhies, 1992).

The scale invariance of the model of equation 1 predicts that loss of homeostasis or dysregulation would occur at other levels of biological organization. We have found loss of silencing of subtelomeric genes during the yeast life span (Kim *et al.*, 1996) that indicates changes in the organization of subtelomeric heterochromatin. Indeed, cytological evidence for such changes has been presented (Kennedy *et al*., 1997). Because there is no telomere shortening during aging in yeast (D'mello and Jazwinski, 1991), it may be possible to extrapolate this loss of silencing to other heterochromatic regions of the genome, the result of which would be age-related gene dysregulation (Jazwinski, 1996). Changes in silencing during the life span may affect aging. A gain of function mutant in the *SIR*4 gene, which encodes a component of a silencing complex, exhibits an extended life span (Kennedy *et al*., 1995). The *CDC*7 gene has been implicated in silencing (Axelrod and Rine, 1991). The effect of transient inactivation of the

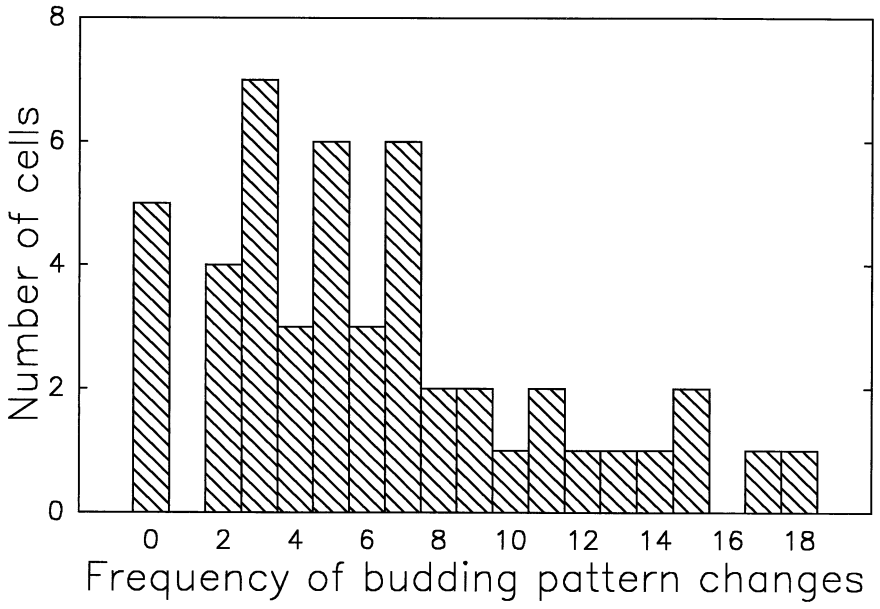

FIG. 4. Budding pattern switching frequency. A histogram of the number of cells in an aging cohort arranged according to the frequency of budding pattern changes before death is shown. The data were derived from the control cells in the experiment depicted in Fig. 3.

Cdc7 protein on yeast longevity (Egilmez and Jazwinski, 1989), suggests that changes in silencing can affect aging.

Changes in silencing might occur at any time during the life span of a yeast cell. However, the yeast cell is clearly most vulnerable during the S phase of the cell cycle following DNA replication when the chromatin must be reassembled. This reassembly encompasses the incorporation of silencing complexes on the chromatin. These events provide amply opportunity for changes in the silencing status of the chromatin of the aging yeast, and they contribute empirical support for the iteration in equation 1 because they take place repeatedly during the life span.

If *RAS*2 acts through factor *A*, this gene could affect the intrinsic level of silencing of subtelomeric heterochromatin. As seen in Fig. 5, the deletion of *RAS*2 resulted in a pronounced reduction in the silencing observed at the right telomere of chromosome V. The reduction of intrinsic silencing on *RAS*2 deletion, described here, would accentuate loss of silencing during yeast aging. The attenuation of *RAS*2 expression with age (Sun *et al*., 1994) may contribute to the loss of silencing normally seen during the yeast life span. Not surprisingly, deletion of *RAS*2 shortens life span (Sun *et al*., 1994). These results provide support for the theoretical construct of equation 1 at the molecular level. There are observations that raise the possibility that loss of organization at the level of heterochromatin domains and gene dysregulation are pertinent to aging in mammals, as they are in yeast (discussed in Jazwinski, 1996).

The studies described in this communication suggest that biological organization, which denotes the integration of pathways and interactions, at various levels from molecular to organismal, plays an important role in determining life span. Decay of this organization leads to loss of homeostasis and is associated with aging. Individuals in an aging population appear to become stratified epigenetically (Jazwinski, 1996), likely due to dysregulation at various

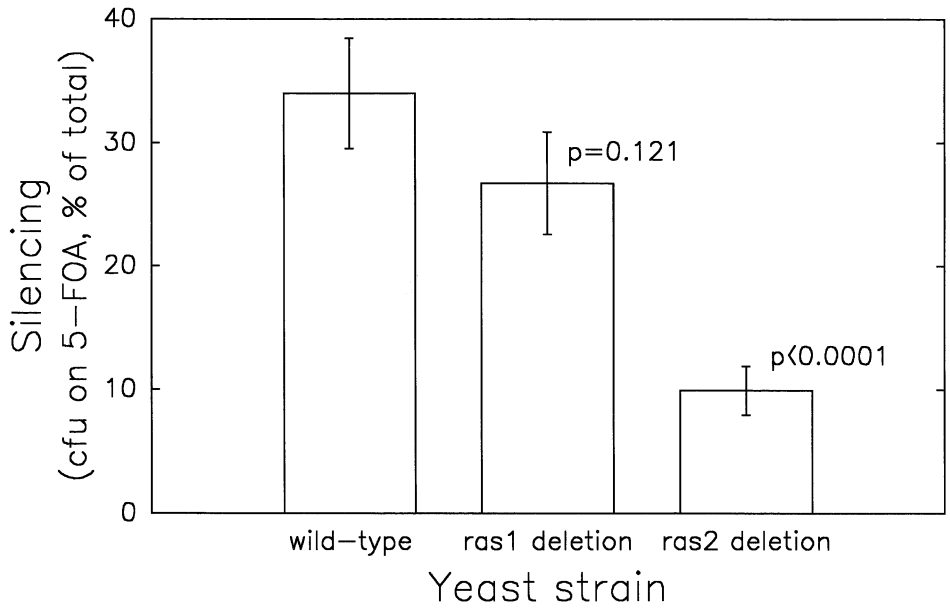

FIG. 5. Effect of *RAS*2 on telomeric silencing. The effects of deleting either *RAS*1 or *RAS*2 on the silencing of a *URA*3 gene adjacent to the telomere at the right arm of chromosome V are shown. Cells in which *URS*3 is silenced can grow on 5-fluoroorotic acid (5-FOA) in the presence of uracil. Results are expressed as the percent of the total viable cells, determined by plating, that form colonies (cfu) on medium containing 5-FOA. The error bars show the standard error ($p = 0.121$ for *ras*1Δ, $p < 0.0001$ for *ras2*Δ).

levels of organization, resulting in changes at the level of the individual (Carey, 1997). These features of the aging process are predicted by equation 1. This equation is applicable to aging generally. It provides a deterministic cause for the randomness of the phenomenology associated with aging, a dynamic and nonlinear process, because it constitutes a defined rule that allows us to determine the state of the system given any initial condition yet provides for the unpredictability of the outcome with respect to the state of any particular, individual aging system.

Furthermore, equation 1 indicates change as a cause rather than a consequence of the aging process. There are other specific predictions of our model at various levels of biological organization that can be readily made in addition to those tested here. All of them share the feature that the bifurcation described by equation 1 is the more distinct the greater the augmentation of the relevant change due to aging. Equation 1 is a simple construct that sustains the properties of aging systems. It can be expanded and refined to provide for additional features of aging. In particular, it will be useful to explore the dynamic qualities of factor *A*.

Acknowledgments—We wish to thank R.L. West, J.W. Curtsinger, S. Pletcher, and W. Johnson for their assistance. This work was supported by grants from the National Institute on Aging of the National Institutes of Health (U.S.P.H.S.). A. Benguria held a fellowship from the Ministry of Education and Culture of Spain.

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