Relation Between the Efficiency of Homothallic Switching of Yeast Mating Type Genes and the Distribution of Cell Types

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Homothallic switching of yeast mating type genes occurs as often as each cell division, so that a colony derived from a single haploid spore soon contains an equal number of *MATa* and *MAT α* cells. Cells of opposite mating types conjugate, and eventually the colony contains only nonmating *MATa/MAT α* diploids. Mutations that reduce the efficiency of homothallic *MAT* conversions yield colonies that still contain many haploid cells of the original spore mating type plus a few recently generated cells of the opposite mating type. These ($a > \alpha$)- or ($\alpha > a$)-mating colonies also contain some nonmating diploid cells. As an alternative to microscopic pedigree-analysis to determine the frequency of mating type conversions in a variety of mutant homothallic strains, we analyzed the proportions of *MATa*, *MAT α* , and *MATa/MAT α* cells in a colony by examining the mating phenotypes of subclones. We developed a mathematical model that described the proportion of cell types in a slow-switching colony. This model predicted that the proportion of nonmating cells would continually increase with the size (age) of a colony derived from a single cell. This prediction was confirmed by determining the proportion of cell types in colonies of an *HO swi1* strain that was grown for different numbers of cell divisions. Data from subcloning ($a > \alpha$) and ($\alpha > a$) colonies from a variety of slow-switching mutations and chromosomal rearrangements were used to calculate the frequency of *MAT* conversions in these strains.

In wild-type homothallic strains of *Saccharomyces cerevisiae*, one mating type (*MAT*) allele can be replaced by the opposite *MAT* allele as often as every cell division (5, 8). A few generations after the germination of a haploid *MATa* or *MAT α* spore, the growing colony contains an equal number of *MATa* and *MAT α* cells, which can conjugate to form a colony containing nonmating *MATa/MAT α* diploid cells. Mutations that reduce the efficiency of homothallic *MAT* conversions result in colonies that have a distinctive, unequal, dual-mating phenotype (1). For example, a homothallic (*HO*) *MAT α* spore carrying the slow-switching mutation (*swi1*) will grow into a colony containing many *MAT α* cells plus a few *MATa* cells, which arise by infrequent switching. This ($\alpha > a$)-mating colony will also contain some nonmating *MAT α /MATa* diploid cells from conjugations of cells of the opposite mating type (1). A variety of mutations that affect the efficiency of *MAT* conversion have been identified: these include *cis*-acting mutations within the *MAT* locus (*MAT α -inc* [9] and *MATa-inc* [6]), *cis*-acting mutations adjacent to *MAT* (*stk1* and *stk2* [4]),

defective alleles of *HO* (6), and an unlinked recessive mutation (*swi1* [1]).

The efficiency of *MAT* conversions can be measured directly by pedigree analysis of homothallic cells (5, 8). The switching of a cell from *MAT α* to *MATa* can be observed microscopically by the response of *MATa* cells to α factor. Such pedigree studies have been used to show that approximately 75% of the wild-type homothallic cells that have undergone one cell division previously switch to the opposite mating type. This efficiency is high enough to generate a nonmating colony. In the case of strains that switch mating type much less efficiently, direct pedigree analysis becomes laborious. For example, if the efficiency of switching of a mutant strain were 2%, one would have to follow 500 eligible cells to observe only 10 switches. The problem of establishing switching efficiencies from single-cell lineages is especially great in cases where switching of *MATa* to *MAT α* must be followed, because each cell must be moved repeatedly away from and then back to a source of α factor (5, 8).

An alternative way to estimate the efficiency

of *MAT* conversions was developed by Haber and Garvik (1). The relative efficiency of switching from one *MAT* allele to the other can be determined by analyzing the relative proportions of *MAT* α , *MATa*, and *MATa/MAT* α cells within a colony. For example, when an ($\alpha > a$) colony derived from a *swi1 HO MAT* α strain is subcloned, the *MAT* α cells present give rise to ($\alpha > a$) colonies, the few *MATa* cells yield ($a > \alpha$) colonies, and the *MAT* α /*MATa* cells yield nonmating colonies. A typical result for *swi1 HO MAT* α strains was that about 90% of the subclones were ($\alpha > a$)-mating, 1% were ($a > \alpha$)-mating, and 10% were nonmating. Thus, *swi1* appeared to reduce the efficiency of *MAT* conversions to about 5 to 10% of the wild-type value.

Although a rough estimate of switching efficiency was obtained by this approach, we wished to develop a more rigorous mathematical treatment to determine the efficiency of switching in a variety of mutant strains. In this paper we describe a model for the growth of slow-switching colonies and equations that describe the proportions of *MATa*, *MAT* α , and *MATa/MAT* α cells that are found as a colony grows. These equations were used to determine the relative efficiencies of homothallic switching in various mutant strains. An experimental test of the model is presented.

MATERIALS AND METHODS

Strains and growth conditions. Strain BW222R-1D (*HO swi1 MAT* α *HML* α *HMRA lys5 ade5*) was constructed by Barbara Wiefenbach. Cells were grown on agar medium containing 1% yeast extract, 2% peptone (Difco Laboratories), and 2% dextrose (YEPA agar) in plates at 30°C. Cells were sporulated on 1% potassium acetate plates at 30°C. Colonies derived from single spores were obtained by micromanipulation of glucanase-treated asci on YEPA agar plates (7).

RESULTS

Distribution of cell types in slow-switching colonies. For the purposes of the discussion below, we assumed that the initial cell of a colony (for example, a spore) was a *MATa* cell that had not divided. Exactly analogous calculations could be performed if the initial cell were *MAT* α . We assumed that homothallic switching followed the rules established by Hicks and Herskowitz (5), namely, that a cell could not switch mating type until after it divided once. Thus, only one-half of the *MATa* cells in a dividing culture were eligible to switch in that generation. We also assumed that any *MAT* α cells that were formed by switching mated with an adjacent *MATa* cell during the next cell

generation. Therefore, the only *MAT* α cells that were detected by a mating test or by subcloning were those which were generated during the last cell division. We also assumed that the mating of two haploid cells to form a *MATa/MAT* α nonmating diploid cell took one generation, so that one *MATa* cell and one *MAT* α cell at the *i*th generation formed one nonmater at the (*i* + 1)th generation. Finally, we assumed that all cell types grew with the same doubling time. Obviously, the coefficient multipliers in the equations could be changed easily if more accurate experimental estimates of mating time lags and relative haploid and diploid growth rates were available. For the analysis below we used the following terms: Na_i = number of *MATa* cells at the *i*th generation; $N\alpha_i$ = number of *MAT* α cells at the *i*th generation; and Nn_i = number of *MATa/MAT* α cells at the *i*th generation.

The number of *MATa* cells in the next generation will be reduced in the following two ways: by mating with preexisting *MAT* α cells to form diploids and by switching to new *MAT* α cells. If there are $N\alpha_i$ *MAT* α cells which can mate at the *i*th generation, the number of *MATa* cells will be depleted to ($Na_i - N\alpha_i$). The remaining population of *MATa* cells is further depleted by the cells which switched mating type. Only one-half of the ($Na_i - N\alpha_i$) *MATa* cells will have divided previously (become "mothers") and therefore are eligible to switch. If the efficiency with which any given cell could convert to *MAT* α is given by *p*, then $1/2(Na_i - N\alpha_i)p$ cells will switch. The *MATa* cells that remain (i.e., those that neither mated nor switched) will then divide to produce two cells in the next generation. Thus:

$$\begin{aligned} Na_{(i+1)} &= 2[(Na_i - N\alpha_i) \\ &\quad - (Na_i - N\alpha_i)p/2] \\ &= (Na_i - N\alpha_i)(2 - p) \end{aligned} \quad (1)$$

By the same logic, the number of *MAT* α cells will be:

$$\begin{aligned} N\alpha_{(i+1)} &= 2[(Na_i - N\alpha_i)p/2] \\ &= p(Na_i - N\alpha_i) \end{aligned} \quad (2)$$

The third cell type (nonmating cells) consist of those nonmaters that already existed (Nn_i) and will double in the next generation and those which will be formed by mating of *MATa* and *MAT* α cells. The number of cells which can mate is determined by the number of the relatively rare *MAT* α cells. Thus:

$$Nn_{(i+1)} = 2Nn_i + N\alpha_i \quad (3)$$

The fraction of each cell type is given simply by the number of cells of one type at a given generation divided by the total number of cells at that generation. Because there is a one-generation lag in the formation of *MATa/MAT α* diploids and two haploid cells combine to form only one diploid cell, the total number of cells is not simply 2^t .

The iterative equations given above could be used to form more explicit exponential equations. The difference expression (for example, $Na_{i+1} - Na_i$) could be approximated to differential equations with respect to time (in generations) and the growth constant K (dNa/dKT). The solutions of these equations yielded expressions for each cell type as sums of exponential terms. The results from this approximation were only slightly different from the results from the iterative method, as follows:

$$Na = \left(\frac{2-x}{1-x} \right) e^{(1-2x)KT} - \left(\frac{x}{1-x} \right) e^{-KT} \quad (4)$$

$$N\alpha = \left(\frac{x}{1-x} \right) e^{(1-2x)KT} - \left(\frac{x}{1-x} \right) e^{-KT} \quad (5)$$

$$Nn = \left[\frac{1}{2(1-x)} \right] [(1-x)e^{KT} + xe^{-KT} - e^{(1-2x)KT}] \quad (6)$$

where T is the number of generations after the two-*a*-cell stage (time after which switching was allowed), K is $\ln 2$, and x equals the efficiency of switching.

Effect of efficiency of *MAT* switching on the proportion of cell types. Using a programmable WANG 500 calculator, we calculated the proportions of cell types for different efficiencies of switching that would have been expected after a number of cell divisions. Our initial conditions were the two *a* cells that were derived from the original cell, one of which was eligible to switch. In practice, strains are analyzed by subcloning (plating dilutions or streaking for many single colonies) only after they grow up as single colonies on the dissection plate containing more than 10^6 cells. In many cases the subcloning is not done until after such colonies are picked from the dissection plate onto a master plate and allowed to grow up again overnight (approximately 11 generations more). Thus, we calculated the proportions of cell types for the following two situations: after 23 generations (approximately the number of divisions to make a small colony from a single cell on a dissection plate) and after 34 generations (the number of generations if the original colony was first restreaked onto a master plate before sub-

FIG. 1. Proportions of *MATa* and *MAT α* haploids and *MATa/MAT α* diploids, as calculated from the equations given in the text, assuming that a colony was derived from a single homothallic *MATa* cell that divided once before switching could begin. The open symbols show the proportions of the three cell types after 34 generations, whereas the solid symbols show the proportions after 23 generations. Symbols: □ and ■, *MATa*; ◇ and ◆, *MATa/MAT α* ; ○, *MAT α* .

cloning from the master plate).

The results of these calculations are shown in Fig. 1. It is evident that for a given non-zero efficiency of switching, the proportion of non-maters increases with increasing numbers of cell divisions. This is to be expected, as the process of switching is assumed to be essentially irreversible; any *MAT α* cell that is formed will mate and become a nonmater in the next generation. It is also evident that we cannot use a subcloning test to distinguish between cells that switch with 100% efficiency and cells that are only 30% as efficient. In fact, after 23 generations 99% of the cells will be nonmaters with an efficiency of switching of 26%, and if a colony is allowed to grow for 34 generations, 99% of the cells will be nonmaters if the efficiency of conversion is only 18%.

Changes in the proportions of cell types during colony growth. As described above, the proportion of nonmating cells in a slow-switching colony will progressively increase during the growth of the colony. As a test of this prediction, we followed the proportions of ($\alpha > \mathbf{a}$), ($\mathbf{a} > \alpha$), and nonmating subclones in colonies derived from *HO swi1 MATa* or *HO swi1 MATa* spores. Individual spores were micro-manipulated onto a YEPD agar plate that had been cut into 1-cm squares. One spore was placed on each square, and the spores were germinated and grown at 30°C. At intervals, agar squares were removed from the plate with a sterile spatula, and the cells were suspended with vigorous mixing in 0.5 ml of sterile water. The cells were then spread onto YEPD plates at appropriate dilutions to determine the total number of colony-forming units in the growing colony. Between 20 and 80 colonies (subclones of the original colony) were picked and tested for mating type. The results are shown in Fig. 2. As predicted, the proportion of ($\mathbf{a} > \alpha$)- or ($\alpha > \mathbf{a}$)-mating colonies decreased with the age (size) of the colony. A scatter of points was expected from the stochastic nature of infrequent switching events; a *MAT* conversion after three or four cell generations produced a colony with many nonmating cells and few *MATa* and *MATa* cells [which grew into ($\mathbf{a} > \alpha$) and ($\alpha > \mathbf{a}$) subclones, respectively]. The line in Fig. 2 shows the ex-

FIG. 2. Proportions of ($\mathbf{a} > \alpha$) and ($\alpha > \mathbf{a}$) subclones in colonies derived from *HO swi1 MATa* or *MATa* spores of strain BW222R-1D grown for varying numbers of cell divisions. Colonies were grown on separate agar blocks. Cells were suspended in sterile water and spread with appropriate dilutions onto YEPD agar plates. Between 20 and 80 subclones from each colony were tested for mating phenotype, and the proportions of ($\mathbf{a} > \alpha$), ($\alpha > \mathbf{a}$), and nonmating colonies were recorded. The proportions of the predominant ($\mathbf{a} > \alpha$) (○) and ($\alpha > \mathbf{a}$) (●) mating types are shown as functions of total colony size (cell number) for each colony. The solid line shows the theoretical result for an efficiency of 0.05 (equation 1).

pected proportions of ($\mathbf{a} > \alpha$) or ($\alpha > \mathbf{a}$) subclones based on an efficiency of *MAT* conversions of 0.05 (equation 1).

DISCUSSION

We developed equations that describe the proportions of haploid and diploid homothallic cells in a growing colony. These equations provide a quantitative basis for determining the relative efficiency of homothallic conversions of *MAT* alleles for a variety of slow-switching mutations. We also examined the efficiency of switching of a number of mutations which we previously described. The efficiencies of *swi1* (1), *MATa-inc* (6), *stk1* and *stk2* (5), and *HO-1* (6) are shown in Table 1. In addition, we examined the efficiency of converting *MATa* to *MATa* when the donor locus *HMLa* was translocated to another chromosome (3). These equations will be useful for determining the changes in the proportions of cell types under various conditions that reduce the efficiency of homothallic mating type switching.

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TABLE 1. Mutations and rearrangements affecting the efficiency of homothallic switching

Mutation or rearrangement	Fraction of subclones ^a		Efficiency of switching (%)
	Nonmating	($\mathbf{a} > \alpha$) colonies	
<i>swi1</i>	0.11	0.88	1.2
<i>MATa-inc</i>	0.24	0.75	2.5
<i>stk1</i>	0.17	0.82	1.9
<i>stk2</i>	0.32	0.67	3.2
<i>HO-1</i> $\alpha \rightarrow \mathbf{a}$	0.48	0.52 ^b	5.0
<i>HO-1</i> $\mathbf{a} \rightarrow \alpha$	0.79	0.21	8.7
<i>HMLa</i> on chromosome XII	0.05	0.95	0.5
<i>HMLa</i> on chromosome T3	0.91	0.09	11.0
Wild type ^c	1.0	0.00	40-75

^a Since the *HMLa* rearrangements and some mutations (*stk1*, *stk2*, *MATa-inc*) do not appreciably inhibit conversions from *MATa* to *MATa*, any *MATa* cells harvested at subcloning appear as nonmating colonies. Therefore, these efficiencies of switching are best estimated from the fraction of ($\mathbf{a} > \alpha$) colonies. About 1% of the subclones from cells with these *cis*-acting mutants are $\mathbf{a}$ -maters, resulting from "illegal" transpositions that replace *HMLa* with *HMLa* (2, 4, 6).

^b Fraction of subclones that are ($\alpha > \mathbf{a}$).

^c From the pedigree experiments of Strathern and Herskowitz (8) and from our own work (Haber, unpublished data), the actual efficiencies of switching in several wild-type strains range from about 40 to 75%.

the differential forms of the equations. Ruthe Wilson helped carry out the experiments.

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