Switching at the cellular level in the white–opaque transition of *Candida albicans*

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The 'white–opaque transition' in *Candida albicans* strain WO-1 provides a unique system for analysing high-frequency switching at the cellular level because of the difference in the budding phenotypes of the white and opaque phases. Single white and opaque cells were placed on agar and monitored for the dynamics of cell division, microcolony genesis and switching to the alternative phase. It is demonstrated that at 24 °C, opaque cells can switch directly to white cells but white cells first generate an elongate, pseudohyphal-shaped precursor in the transition to an opaque cell. Cells in either phase can generate a daughter cell in the alternative phase, then revert immediately to the genesis of subsequent daughter cells in the original phase. By developing a mathematical model for switching at the cellular level which subtracts mother cells and switched daughter cells from the pool of switching candidates, the probability for an opaque cell to generate a white daughter cell in any single generation was calculated to be 1.0×10^{-1} , and the probability for a white cell to generate an opaque daughter cell in any single generation was calculated to be 1.7×10^{-5} at 24 °C on nutrient agar. The mean number of generations before an opaque cell generated a white daughter cell was calculated to be 3.4 and the mean number before a white cell formed an opaque cell was calculated to be 15.8 at 24 °C on nutrient agar. Finally, high-temperature induction of the opaque to white transition was analysed at the cellular level and demonstrated to involve frequent bipolar formation of white daughter cells on the original opaque mother cell, and in some cases intermediate phenotypes.

Introduction

Most strains of *Candida albicans* and the related species *Candida tropicalis* are capable of switching at high frequency between an apparently limited number of general phenotypes distinguishable by colony morphology (Slutsky *et al.*, 1985, 1987; Pomes *et al.*, 1987; Soll *et al.*, 1987, 1988). The frequencies of switching have been indirectly determined by analyses of colony morphologies in clonal plating experiments (Slutsky *et al.*, 1985, 1987; Anderson & Soll, 1987), or in a more direct fashion in liquid microcultures by the Luria–Delbruck fluctuation formula (Rikkerink *et al.*, 1988). However, switching has previously not been described at the cellular level. The 'white–opaque transition' in *C. albicans* strain WO-1 provides us with a unique opportunity to observe switching at the cellular level because of the distinct differences in cellular morphologies distinguishable by standard light microscopy (Slutsky *et al.*, 1987; Anderson & Soll, 1987; Anderson *et al.*, 1989). In the white phase, budding cells are relatively round and resemble most *C. albicans* strains in the budding phenotype. In contrast, in the opaque phase, budding

cells are elongate or bean-shaped, and roughly twice the volume of white cells (Slutsky *et al.*, 1987). At the ultrastructural level, opaque cells differ from white cells in a number of characteristics, including the presence of a large vacuole filled with membranous or spaghetti-like material (Slutsky *et al.*, 1987; Anderson *et al.*, 1990), and opaque-specific cell-wall pimples (Anderson & Soll, 1987; Anderson *et al.*, 1989, 1990).

In the present study we have used the difference in shape of white and opaque cells to examine the cellular dynamics for a switch from white to opaque and from opaque to white, to estimate the probability of switching in both directions in microcolonies generated from single cells, and to visualize the transition from opaque to white after temperature induction. A mathematical model is developed to calculate probabilities of switching.

Methods

Maintenance of stock cultures of strain WO-1. *C. albicans* strain WO-1 was isolated from the blood and lungs of a bone-marrow transplant patient (Slutsky *et al.*, 1987). Stock cultures of the strain were maintained on agar slants in capped tubes at room temperature. For

experimental purposes, cells from the original stock slants were clonally plated on agar containing the defined nutrient medium of Lee *et al.* (1975) supplemented with $70 \mu\text{g}$ arginine ml^{-1} and $0.1 \mu\text{M}$ -ZnSO₄ (Bedell & Soll, 1979). White and opaque colonies were distinguished by colour and shape according to methods previously described (Slutsky *et al.*, 1987).

Single cell analysis. Cells from a white or an opaque colony were removed by scraping a culture loop across the colony and spreading the cells on nutrient agar. Individual cells were then removed from the agar with a glass needle connected to a micromanipulator attached to the frame of a Zeiss microscope, and placed individually on nutrient agar either in a Petri dish or on a microscope slide. Cells and microcolonies were photographed on T-Max 100 film (Eastman Kodak) at time intervals with a Zeiss ICM 405 inverted microscope equipped with a green filter.

Switching frequency analysis. Opaque cells were removed from an opaque colony, diluted with distilled water to a concentration of 10^5 c.f.u. ml^{-1} , and 0.1 ml cell suspension was spread in the centre of an agar plate. These cultures were incubated for 12 to 16 h at 24 °C, and each microcolony then scored for the number of white and the number of opaque cells. White cells were removed from a white colony, diluted with distilled water to a concentration of 10^5 c.f.u. ml^{-1} , and 0.1 ml spread on an agar plate. These cultures were incubated for 17 to 20 h at 24 °C, and each microcolony then scored for the number of white and the number of opaque cells. When microcolonies grew beyond 50 cells, they were teased apart into groups containing 10 to 15 cells before counting cell types.

Results

The development of opaque or white microcolonies from single cells

To define the dynamics of cell division in the early genesis of an opaque colony at 25 °C, opaque cells were individually placed on nutrient agar. Examples of the division dynamics of two such cells are presented in Fig. 1, in which the original mother cells are indicated with arrows. For the cell in Fig. 1(a), a half-grown daughter cell was evident at 3 h of incubation. At 5 h of incubation, a daughter cell was evident on the mother cell at the opposite pole, and the original daughter cell had formed an apical bud. The cell number doubled on average every 2 h between 3 and 14 h of incubation. As the microcolony developed, cells shifted so that little if any space was left in the colony proper. For the cell in Fig. 1(b), roughly the same dynamics, including bipolar budding, were evident. In these two examples of opaque microcolony development, all divisions resulted in opaque cell phenotypes. The development of more than 100 opaque colonies from single opaque cells was monitored with similar results.

Examples of the division dynamics of two white cells placed on agar at 24 °C are shown in Fig. 2, in which the original mother cells are again indicated with arrows.

For the cell in Fig. 2(a), a half-grown daughter cell was evident at 3 h of incubation. At 5 h of incubation, a daughter cell was evident on the mother cell, but at the same pole as the original daughter cell, and the original daughter cell had formed a bud. The cell number doubled on average every 2 h between 3 and 14 h of incubation. For the cell in Fig. 2(b), which already had a small bud at the time of isolation, roughly the same division dynamics were observed. Although white and opaque microcolonies divided at approximately the same rate and contained approximately the same number of cells after 14 h, white cells never exhibited bipolar budding and white microcolonies were approximately half the diameter of opaque microcolonies, presumably a result of the smaller diameter of the component white cells (Slutsky *et al.*, 1987). The development of more than 100 white colonies from single white cells was monitored with similar results.

The transition from opaque to white at the single cell level

To observe a switch from opaque to white at the cellular level, opaque cells (100 cells per experiment; three experiments) were plated on nutrient agar at 24 °C and individually monitored for a spontaneous switch from opaque to white at the first evagination. The opaque cell in Fig. 3(a) already had a small round bud at the time of isolation. After 3 h of incubation, the daughter cell had expanded into a white cell, and a cluster of five additional white cells was localized at the original white daughter cell position. After 4.5 h of incubation, the microcolony consisted of roughly 13 white cells and the original opaque cell, representing a mean generation time of less than 2 h. It should be noted that the opaque cell in Fig. 3(a) failed to release opaque daughter cells even though it was phenotypically opaque. No intermediate phenotype was evident in the opaque to white transition at 24 °C, and all progeny in the microcolony at 9 h exhibited the small, round, white phenotype. Twenty-six switches from opaque to white were observed with similar dynamics.

In two cases, an opaque cell gave rise to both white and opaque daughter cells. In Fig. 3(b), the isolated opaque cell formed a white bud by 6 h of incubation. At 7.5 h of incubation, it had formed an opaque daughter cell at the same time that the white daughter cell had formed a white bud. At 9 h of incubation, the microcolony consisted of half white cells and half opaque cells. This scenario demonstrates that an opaque cell can generate a white daughter cell, then revert to the genesis of an opaque daughter cell. Again no intermediate phenotype was evident in the opaque to white switch.

Fig. 1. The development of opaque microcolonies from single opaque cells. Individual opaque cells (a, b) were placed on nutrient agar and incubated at 24 °C. The incubation time in hours is shown in the upper left-hand corner of each photograph. The arrows indicate mother cells. Bars, 10 μm .

Fig. 2. The development of white microcolonies from single white cells. Individual white cells (*a, b*) were placed on nutrient agar and incubated at 24 °C. The incubation time in hours is shown in the upper left hand corner of each photograph. The arrows indicate mother cells. Bars, 10 μ m.

Fig. 3. Switching from opaque to white at the cellular level. (a) An opaque cell giving rise solely to white cells. (b) An opaque cell giving rise first to a white cell, then to an opaque cell. Individual opaque cells were placed on agar at 24 °C and scanned for white cell genesis. Incubation time in hours is shown in the upper left hand corner of each photograph. The arrows indicate mother cells. Bars, 10 µm.

The transition from white to opaque at the single cell level

Far fewer examples of white cells forming opaque daughter cells were observed at the single cell level since it has been demonstrated to be a far rarer event (Rikkerink *et al.*, 1988; also, see later section on switching frequencies). To observe a switch in the white to opaque direction, 10000 cells were placed on nutrient agar in each of five experiments and monitored for opaque cell formation. The white cell in Fig. 4(a) had already generated an elongate, pseudohyphal-shaped cell which in turn had formed a second elongate cell by 11 h of incubation. These daughter cells were far more elongate than opaque cells and were bulbous at their base (compare elongate daughter cells in Fig. 4(a) with standard opaque cell morphology in Fig. 1). The shapes of these intermediate cell types were similar to white cells which have been induced to form a hypha early in the budding cycle (Buffo *et al.*, 1984; Soll *et al.*, 1985): relatively round at the position of the original daughter bud and tapering apically into a hyphal or pseudohyphal form (Soll *et al.*, 1985). In addition to the elongate daughter cell, the mother cell in Fig. 4(a) had also generated two white daughter cells by 11 h. The elongate progeny continued to generate elongate, pseudohyphal-shaped cells until 17 h. After 17 h, opaque cells formed apically and laterally from the original elongate daughter cells. By 21 h, the microcolony consisted of a tightly packed white sector and a less tightly packed opaque sector emanating from the original, branched cells. The formation of an elongate, pseudohyphal-shaped daughter cell prior to opaque cell formation was observed in 21 cases for switches from the white to opaque phenotype.

The white cell in Fig. 4(b) had already generated an elongate daughter cell with an apically tapered phenotype which in turn had generated an opaque-shaped daughter cell by 12.5 h of incubation. The original elongate cell gave rise to one or more daughter cells with apparently the opaque phenotype, but the phenotype of all subsequent progeny was white. Additional daughter cells generated by the white cells were also white. By 21 h, the microcolony consisted of an apparently opaque cell core with a majority of peripheral white cells.

The white cell in Fig. 4(c) had already generated an elongate tapered cell by 11 h of incubation. This daughter cell continued to grow as a relatively wide hypha until 14 h of incubation. After 17 h of incubation, opaque cells were generated laterally and apically from the original hypha.

Switching frequencies measured in microcolonies

The capacity to distinguish between white and opaque cell phenotype at the cellular level provided us with a method for estimating switching frequencies in microcolonies. Individual cells were plated on nutrient agar and incubated for 12 to 24 h at 24 °C. Each microcolony was then scored for the number of white and opaque cells.

In Table 1(a), results are presented for switching from opaque to white in opaque microcolonies. Seven individual experiments were done in which a total of 645 microcolonies, including 9624 cells, were scored. The average colony contained 16 cells. The frequency of a colony containing one or more white cells was 2.5×10^{-1} . The proportion of white cells in all cells scored was 1.2×10^{-1} .

In Table 1(b), results are presented for switching from white to opaque. Ten individual experiments were done in which a total of 17441 microcolonies, including 2083160 cells, were scored. The average colony contained 119 cells. Microcolonies larger than 50 cells were teased apart into groups of 10 to 15 cells before cells were scored for phenotype. The frequency of a colony containing one or more opaque cells was 4.4×10^{-3} . The number of cells exhibiting the intermediate, pseudohyphal phenotype ranged between one and three in colonies containing a switch. These intermediate cell types were not scored as either white or opaque. The proportion of opaque cells for all cells scored was 5.3×10^{-4} . These results demonstrate that the frequency of switching from opaque to white is far greater than that from white to opaque in microcolonies on defined nutrient agar at 24 °C.

Mathematical model for determining the probability of switching and estimating the mean number of generations preceding a switch

To determine the probability of a switch from one phase (A) to the other (B) at the cellular level, a mathematical model was developed. The assumptions of the model are that at 24 °C (1) the mother cell (exhibiting the phenotype of phase A) itself cannot change its phenotype (change its shape to phase B), (2) when a daughter cell expresses the alternative phenotype (phase B), all progeny will show the switched phenotype (phase B), and (3) each daughter cell has a probability of expressing the alternative phenotype (phase B) when formed. At generation 0, there are M mother cells (in phase A). At

Fig. 4. Switching from white to opaque at the cellular level. Individual white cells were placed on agar at 24 °C and scanned for opaque cell genesis. (a) A white cell giving rise to elongate cells which in turn give rise to an opaque cell sector (note that the white cell continues to give rise to white cells, which form a white cell sector); (b) a white cell which gives rise to elongate cells which in turn give rise to opaque cells which rapidly revert to white cells; (c) a white cell which gives rise to a hypha which in turn gives rise to opaque cells. Bars, 10 µm.

Table 1. *Switching frequencies and the calculated probability of switching in the opaque to white and white to opaque direction determined from the phenotypic composition of microcolonies*Formulae for the weighted means α_{Exp} and α_M are derived in the text.(a) *Opaque to white transition*

Experiment	Incubation time (h)	No. of microcolonies	Total no. of cells	No. of white cells	Proportion of white cells	Proportion of colonies with one or more white cells	Weighted mean α_{Exp} for each experiment	Weighted mean $\alpha_{\text{Exp}} \times \text{total no. of cells}$
1	14	105	930	71	7.6×10^{-2}	1.6×10^{-1}	8.8×10^{-2}	81.8
2	17	86	2004	31	1.5×10^{-2}	9.0×10^{-2}	5.4×10^{-3}	10.8
3	16	82	1504	298	2.0×10^{-1}	3.1×10^{-1}	1.8×10^{-1}	270.7
4	12	89	656	92	1.4×10^{-1}	2.4×10^{-1}	1.8×10^{-1}	118.1
5	15	94	1354	304	2.2×10^{-1}	2.5×10^{-1}	1.8×10^{-1}	243.7
6	13	97	1510	233	1.5×10^{-1}	3.7×10^{-1}	1.1×10^{-1}	166.1
7	12	92	1666	119	7.0×10^{-2}	3.0×10^{-1}	6.1×10^{-2}	101.6
		Totals: 645	9624	Means: 1.2×10^{-1}		2.5×10^{-1}	Total: 992.8	

Probability of a switch from opaque to white (α_M) = $992.8/9624 = 1.0 \times 10^{-1}$

(b) *White to opaque transition*

Experiment	Incubation time (h)	No. of microcolonies	Total no. of cells	No. of opaque cells	Proportion of opaque cells	Proportion of colonies with one or more opaque cells	Weighted mean α_{Exp} for each experiment	Weighted mean $\alpha_{\text{Exp}} \times \text{total no. of cells}$
1	19	127	18790	3	1.6×10^{-4}	2×10^{-2}	7.1×10^{-5}	1.3
2	17	136	14552	3	2.0×10^{-4}	7×10^{-3}	6.2×10^{-5}	9.0
3	17	94	2430	4	1.6×10^{-3}	1×10^{-2}	7.4×10^{-4}	1.8
4	19	119	6307	0	—	—	—	—
5	18	109	4796	0	—	—	—	—
6	24	63	6300	0	—	—	—	—
7	17	177	6372	21	3.3×10^{-3}	6×10^{-3}	1.8×10^{-3}	11.2
8	20	116	6380	0	—	—	—	—
9	17	9200	1343200	20	1.5×10^{-5}	4.4×10^{-4}	4.2×10^{-6}	5.6
10	17	7300	674033	21	3.1×10^{-5}	8.2×10^{-4}	9.9×10^{-6}	6.6
		Totals: 17441	2083160	Means: 5.3×10^{-4}		4.4×10^{-3}	Total: 35.5	

Probability of a switch from white to opaque (α_M) = $35.5/2083160 = 1.7 \times 10^{-5}$

generation 1, each mother cell produces a daughter cell with a probability α of expressing the phenotype of phase B. The number of daughter cells expressing phase B will therefore be αM and the number of unswitched daughter cells (in phase A) will be βM , where $\beta = 1 - \alpha$. After generation 1, the number of unswitched cells (in phase A) is $M + \beta M$, or $(1 + \beta)M$ out of a total cell population of $2M$. Therefore, the population after generation 1 contains $M + \beta M$ mother cells in phase A which are capable of generating a daughter cell in phase B with a probability of α . After generation 2, each of the $2M$ cells in the population generates a daughter, yielding (2) ($2M$) cells, or $2^2 M$ cells. The number of unswitched cells (in phase A) in generation 2 is the original number of mother cells plus the number of unswitched daughter cells:

$$[(1 + \beta)M] + \beta[(1 + \beta)M] = (1 + \beta)^2 M$$

Continuing in this manner, the number of unswitched cells (in phase A) after K generations is $(1 + \beta)^K M$, and

the number of switched cells (in phase B):

$$[2^K - (1 + \beta)^K]M$$

Experimentally, the following can be determined: M , the original number of cells (in phase A); T , the final number of cells (in phase A and B); and S , the final number of switched cells (in phase B). Since $T = 2^K M$, the number of generations can be determined by the formula:

$$K = \frac{\ln T - \ln M}{\ln 2}$$

Once K is calculated, β can be determined by the formula:

$$\beta = \left(\frac{T - S}{M} \right)^{1/K} - 1$$

Since $\alpha = 1 - \beta$, α can be determined by the formula:

$$\alpha = 2 - \left(\frac{T - S}{M} \right)^{1/K}$$

In each experiment, T , α and β were calculated for each microcolony. In a single experiment in which a number of microcolonies were analysed, the mean probability for the experiment, α_{Exp} , was calculated from individual α values weighted for final microcolony size (i.e. final number of cells in each microcolony). The formula for α_{Exp} was:

$$\alpha_{\text{Exp}} = \frac{\alpha_1 T_1 + \dots + \alpha_n T_n}{T_1 + \dots + T_n}$$

where n is the number of microcolonies, α_i ($1 \leq i \leq n$) is the probability of switching in each microcolony, and T_i ($1 \leq i \leq n$) is the final number of cells in each microcolony. Finally, the mean probability α_M for a number of experiments was determined by computing a weighted mean of the α_{Exp} values using the final number of cells in each experiment as the weights. The computations of α_M for opaque to white from the seven independent experiments done, and for white to opaque from the 10 independent experiments done, are presented in Table 1. The probability, α_M , for an opaque to white switch was calculated to be 1.0×10^{-1} , and for a white to opaque switch 1.7×10^{-5} .

The probability α can now be used to estimate the mean number of generations preceding a switch. Beginning with a single mother cell, the number of potential switch events, E , required to produce a single switch must satisfy the following equation:

$$\alpha E = 1$$

Therefore, on average $1/\alpha$ potential switch events must occur before the first switch. To determine the number of generations, K , required to give $1/\alpha$ potential switch events, consider the following family tree in which we assume no switches have yet occurred (Fig. 5). As the result of a division, the cell on the right is always considered the potentially switched daughter cell (X marks the potential switch event). Up to generation K , there are

$$2^0 + 2^1 + \dots + 2^{K-1} = 2^K - 1$$

potential switch events. Hence:

$$2^K - 1 = 1/\alpha, \text{ and } K = \frac{\ln(1/\alpha + 1)}{\ln 2}$$

where K is the expected number of generations before the first switch. Using this formula, the expected number of generations before an opaque cell switches was calculated to be 3.4, and the expected number of generations before a white cell switches was calculated to be 15.8 at 24 °C.

An analysis of temperature-stimulated switching at the cellular level

Rikkerink *et al.* (1988) demonstrated that incubation of opaque cells in nutrient broth for at least 5 h stimulated mass conversion to white colony-forming units. To examine temperature-induced switching at the cellular level, we first tested whether opaque cells incubated individually on agar at 37 °C also mass converted to white cell formation. Sixty individual opaque cells were incubated on agar at 24 °C and sixty on agar at 37 °C for 24 h. All of the 56 colonies generated at 24 °C (four original cells did not divide) were opaque and all of the 57 colonies generated at 37 °C (three original cells did not divide) were white. When 30 cells from 15 colonies generated at 24 °C were in turn plated at 24 °C, they generated opaque colonies, and when 30 cells from 15 colonies generated at 37 °C were in turn plated at 24 °C, the subsequent colonies remained white. Therefore, incubation at 37 °C also stimulated switching *en masse* from opaque to white.

To test whether temperature stimulation of the opaque-white transition on agar exhibited the lag period of roughly 5 h demonstrated in liquid culture (Rikkerink *et al.*, 1988), opaque cells were individually plated at 37 °C on nutrient agar, and at time intervals, cells from the developing microcolonies were separated by micro-manipulation, incubated at 24 °C for 42 h, and the resulting microcolonies scored for phenotype. The results

Fig. 5. Family tree for determining the number of generations, K , required for $1/\alpha$ potential switch events. As division occurs, the cell on the right is always considered the potentially switched daughter cell (X marks the potential switch event), and the cell on the left is always considered the mother cell.

Fig. 6. Examples of induced switching from opaque to white at 37 °C. Opaque cells from 24 °C cultures were transferred to nutrient agar at 37 °C and incubated for the number of hours indicated. (a) An opaque cell which first gives rise to opaque cells up to 4.5 h, then gives rise to intermediate shaped cells after 4.5 h (note the multiple bipolar budding pattern at 4.5 h); (b) an opaque cell giving rise immediately to white cells. The arrows indicate original opaque mother cells. Bars, 10 µm.

on agar were similar to those obtained in liquid culture (Rikkerink *et al.*, 1988). When opaque cells were incubated for less than 5 h at 37 °C, the majority of cells removed from the microcolonies formed opaque colonies at 24 °C. However, after 6 h of incubation at 37 °C, 60% of the cells formed opaque colonies and 40% white colonies, and after 7 h, 100% of the cells formed white colonies.

In Fig. 6, examples are presented of the most common pattern of individual opaque cells switching to white after a shift from 24 °C to 37 °C. In Fig. 6(a), the cell had already evaginated at the time of isolation. By 3 h, the original daughter cell had grown into an opaque cell and the original mother cell had generated a second opaque daughter cell at the opposite cell pole, plus two more evaginations, one at either pole. Between 4.5 and 6 h, most of the new daughter cells generated by the original mother cell and the initial daughter cells exhibited a phenotype which was intermediate between the round white cell and the large asymmetric bean-shaped opaque cell. By 9 h, the microcolony appeared as an inner core of opaque cells surrounded by cells of intermediate phenotype and phenotypically white cells at the microcolony edge. This general scenario, including bipolar budding, was observed during development of the majority of more than 100 microcolonies generated from single opaque cells at 37 °C.

In a less common scenario (Fig. 6b) an existing evagination grew into a white cell prior to 5 h of incubation, and all subsequent daughter cells generated from the original mother cell and daughter cells were white. By 9 h, the microcolony consisted of the original opaque cell surrounded by a shell of white cells. Again the original opaque cell formed frequent buds at opposing cell poles.

To test the phenotypic stability of cells in a microcolony generated from an opaque cell at 37 °C, a single opaque cell was isolated from an opaque colony at 24 °C, and incubated on agar at 37 °C. When the colony contained roughly 50% white cells and 50% opaque cells (after 6 h), every cell in the microcolony was separated, plated individually on nutrient agar, photographed, incubated at 24 °C for 42 h and assessed for colony phenotype. Of the 27 microcolonies generated from phenotypically opaque cells, 17 (77%) were exclusively opaque and 5 (23%) were primarily white. Of the 29 microcolonies generated from phenotypically white cells, 25 (86%) were exclusively white and 4 (14%) were exclusively opaque. Of four microcolonies generated from cells of intermediate phenotype, three were opaque and one was white. These results suggest a small degree of phenotypic instability in both directions for cells in an early microcolony emanating from an opaque cell at 37 °C.

Discussion

The difference in the shape of budding cells in the two phases of the white–opaque transition in *C. albicans* strain WO-1 (Slutsky *et al.*, 1987; Anderson & Soll, 1987; Anderson *et al.*, 1989, 1990) has provided us with an opportunity to examine high-frequency switching at the cellular level. Our results suggest that both the sequence of events and frequency of the transition differ in the opposing directions. At 24 °C, opaque cells were found to form first-generation daughter cells with the white phenotype, whereas white cells were found to produce elongate, tapered daughter cells reminiscent of early budding cells induced to convert to the hyphal growth form (Buffo *et al.*, 1984; Soll *et al.*, 1985). These elongate, tapered cells then generated opaque cells both laterally and apically. When opaque cells were induced to convert *en masse* to white colony-forming units by high temperature induction (Rikkerink *et al.*, 1988), the majority generated cells with phenotypes intermediate between the large bean shape of opaque and the small round shape of white.

The results obtained in this study also suggest a degree of phenotypic instability in early divisions following a switch in both the opaque to white and white to opaque directions at 24 °C, and during high-temperature induction of opaque to white. In a few instances at 24 °C, opaque mother cells produced first a white daughter cell which continued to generate white cells, and then an opaque daughter cell which continued to generate opaque cells. Since a reasonable degree of multinucleation exists in an opaque cell population at 24 °C (Slutsky *et al.*, 1987), it is impossible to distinguish in these cases whether the observed instability is due to a switch in only one of two nuclei, or whether nuclear segregation of the white and opaque trait has occurred at division. Conversely, a few white mother cells at 24 °C gave rise to the elongate morphology found to precede opaque cell formation, but these elongate daughter cells in turn produced white cells. In a few other cases, white cells at 24 °C formed elongate daughter cells which gave rise to opaque cells, but the original white mother cell then formed new white daughter cells. Since white cell populations at 24 °C are almost exclusively uninucleate (Slutsky *et al.*, 1987), these results suggest nuclear segregation of white and opaque at division. When cells were removed from a temperature-induced microcolony of opaque cells after 5 h at 37 °C, a minority of white-appearing cells gave rise to opaque colonies. Therefore, both at 24 °C and 37 °C, cells which have apparently switched at the cellular level may not be fully committed, and are therefore capable of reverting. Since a switch from white to opaque involves several changes in gene expression discernible either by two-dimensional gel

electrophoresis (Soll *et al.*, 1990), or with antisera generated against opaque cells (Anderson *et al.*, 1990), it is possible that instability may involve incomplete activation of opaque-specific genes. This possibility is now being tested.

In the original descriptions of the white-opaque transition, frequencies of switching at 24 °C were inferred from plating experiments in which the population of white colony-forming units in mature opaque colonies was measured to be roughly 2×10^{-2} and opaque colony-forming units in mature white colonies roughly 3×10^{-3} (Slutsky *et al.*, 1987; Anderson & Soll, 1987). However, these frequencies were poor indicators of the rate of switching because of differences in crowding dominance, cohesion (Kennedy *et al.*, 1988), phenotypic instability and cell size and shape (Slutsky *et al.*, 1987). Rikkerink *et al.* (1988) obtained a more direct measure of rate by isolating single cells on agar, then inoculating each agar block supporting a cell into 100 µl of nutrient medium. After 24 h at 25 °C, cells from each microculture were plated on agar and incubated an additional 4 d. By measuring the number of cultures without a switch, they estimated the frequency of switching in the white to opaque direction at 25 °C to be 10^{-4} to 10^{-5} , and in the opaque to white directions 5×10^{-4} , using the Luria-Delbruck fluctuation formula. Although this method was superior to the original plating experiments (Slutsky *et al.*, 1987; Anderson & Soll, 1987), it still did not provide a direct measure of rate at the cellular level since it depended upon colony phenotypes many generations after the switching event. For this reason, we have utilized the difference in cellular phenotype and a simple mathematical model to obtain a measure of switching probability at the cellular level. For microcolonies at 24 °C, we have measured the probability α_M of a switch in a single generation to be 1.0×10^{-1} in the opaque to white direction, and 1.7×10^{-5} in the white to opaque direction. The ratio of the two probabilities is 5882. The expected number of generations, K , before a switch from opaque to white was calculated from α_M to be 3.4, and from white to opaque, 15.8. Because switching frequency in the white-opaque transition is affected by several environmental conditions including ultraviolet irradiation (Slutsky *et al.*, 1985; Morrow *et al.*, 1989), temperature (Rikkerink *et al.*, 1988) and pH (M. S. Bergen and D. R. Soll, unpublished), the probabilities calculated by our model are specific to the conditions of culturing at 24 °C employed in this study.

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