

SEXUAL CONJUGATION IN YEAST

Cell Surface Changes in Response to the Action of Mating Hormones

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ABSTRACT

In the yeast *Saccharomyces cerevisiae*, sexual conjugation between haploid cells of opposite mating type results in the formation of a diploid zygote. When treated with fluorescently labeled concanavalin A, a zygote stains nonuniformly, with the greatest fluorescence occurring at the conjugation bridge between the two haploid parents. In the mating mixture, unconjugated haploid cells often elongate to pear-shaped forms ("shmoos") which likewise exhibit asymmetric staining with the most intense fluorescence at the growing end. Shmoo formation can be induced in cells of one mating type by the addition of a hormone secreted by cells of the opposite mating type; such shmoos also stain asymmetrically. In nearly all cases, the nonmating mutants that were examined stained uniformly after incubation with the appropriate hormone. Asymmetric staining is not observed with vegetative cells, even those that are budded. These results suggest that, before and during conjugation, localized cell surface changes occur in cells of both mating types; the surface alterations facilitate fusion and are apparently mediated by the hormones in a manner that is mating-type specific.

KEY WORDS yeast · mating hormones ·
mannan · concanavalin A · conjugation ·
cell surface · *Saccharomyces cerevisiae*

Sexual conjugation between two haploid yeast cells is a complex process which involves intercellular recognition, mutual interaction, and eventual fusion culminating in the formation of a diploid zygote. Mating is heterosexual, in that **a** and α haploid cells, the two mating types in *Saccharomyces cerevisiae*, mate only with each other. During the conjugation process, **a** and α cells agglutinate to form aggregates of several cells, within which the diploid zygotes form. These events occur optimally (perhaps only) between cells in

the G1 stage of the cell cycle (5, 12, 21). In mating mixtures, the duration of G1 in cells of one mating type can be increased through the action of one (or more) diffusible hormonelike substance(s) produced by cells of the opposite mating type; α cells secrete α -factor, an oligopeptide that affects **a** cells (3, 8, 9), and **a** cells produce **a**-factor, a protease-sensitive substance that acts upon α cells (2, 29). During mating factor-induced G1 arrest (when, of course, nuclear DNA replication cannot be initiated [12]), general RNA and protein syntheses continue (2, 25). Furthermore, with α -factor-treated **a** cells, it has been demonstrated that cell wall synthesis and mitochondrial DNA replication likewise are not

blocked (14, 20). After approximately one generation time of G1 arrest, the cells develop an elongated or pear-shaped appearance and have been termed "shmoos" (15). Similar elongated forms are also observed in mating mixtures.¹

Because the cell wall is the critical site of adhesion during conjugation, information concerning the aspects of its composition and synthesis that are related to mating is a requisite for understanding the process of cell fusion. Little is known about the wall chemistry of zygotes, but Lipke et al. (14) have reported that the morphological changes which occur in **a** cells upon treatment with α -factor require *de novo* synthesis of wall polymers that can be inhibited by sugar analogues or cycloheximide. Their analysis of total wall composition in such cells indicated a higher glucan and lower mannan content compared with that of untreated asynchronously growing **a** cells. In addition, the mannan polysaccharide from treated cells appeared to have fewer long side chains and an increased proportion of unsubstituted mannosyl residues in the backbone.

To assess the biological significance of these quantitative and structural changes in the wall polymers, it is important to know whether the modifications occur throughout the wall matrix or are restricted to a specific region of it. Ultrastructural studies suggest that the latter may be the case; in an **a** shmoo, the wall at the tip of the elongation is thinner and more diffuse than that surrounding the remainder of the treated cell (14), and the walls between pairs of conjugating haploid cells decrease in thickness during the fusion process (19). As a probe for modified regions in the cell wall, we have used concanavalin A labeled by reaction with fluorescein isothiocyanate (FITC-Con A). This lectin is a specific reagent for the mannan on the surface of *Saccharomyces* cells (26, 27). We have found that localized alterations are evident in zygotes as well as shmoos of both mating types and that hormone action is sufficient to induce the formation of these modified areas.

MATERIALS AND METHODS

Yeast Strains and Culture Conditions

The strains of *S. cerevisiae* used in this study are listed with their genotypes in Table I. X2180-1A and X2180-

¹ As discussed previously (7), the convenient term "shmoo" is used because it denotes the shape of the cell without implying that such an extremely altered cell necessarily participates in the mating process.

1B are mating type **a** and α strains, respectively, that are otherwise isogenic. They were derived from X2180 by ascus dissection; all three strains were obtained from the Yeast Genetics Stock Center (Berkeley, Calif.). Strains XJ24-17a and XY505-18C were kindly provided by I. Herskowitz (Eugene, Oregon) and J. Lemontt (Oak Ridge, Tenn.), respectively. The sterile α mutants were originally derived from XT1172-S245c, and the nonmating **a** strains were isolated from XT1177-S47c (15, 16).

Cells were grown in a chemically defined medium (28) containing 0.67% yeast nitrogen base (Difco Laboratories, Detroit, Mich.) without amino acids, 2% glucose and, when necessary, the following supplements: adenine, 30 mg/liter; arginine, 30 mg/liter; histidine, 20 mg/liter; leucine, 40 mg/liter; lysine, 40 mg/liter; methionine, 20 mg/liter; tryptophan, 30 mg/liter; uracil, 20 mg/liter. Unless noted otherwise, cultures were incubated at 30°C on a rotary shaker at ~200 rpm.

Mating Hormones

The Amberlite CG50 fraction of α -factor was used throughout (9). **a**-Factor was prepared by chromatography on phosphocellulose and assayed as described by Betz et al. (2).

Treatment of Cells with Mating Hormones

Exponentially growing cells were transferred to fresh medium containing 2–4 U/ml of the appropriate mating hormone to yield a final concentration of 10^7 cells/ml. The cultures were incubated at 30°C (unless noted) with shaking (200 rpm), and samples of 5×10^6 cells were removed at various times for staining with FITC-Con A.

Zygote Formation

Separate cultures of **a** and α cells growing exponentially were each diluted in fresh medium to 10^7 cells/ml, then mixed in equal amounts and incubated at 30°C with shaking. After 4 h when numerous zygotes could be seen by light microscopy, samples of 5×10^6 cells and zygotes were removed for staining.

Staining with FITC-Con A

The preparation of FITC-Con A has been described previously (26). Before exposure to the conjugate, the yeast were washed with 0.15 M NaCl and resuspended at a concentration of 2×10^7 cells/ml. The procedure of Tkacz et al. (26) was employed for staining the cells and removing unbound FITC-Con A, but the concentration of NaCl in all solutions was reduced to 0.15 M. To inhibit the specific interaction of Con A with the surface mannan of the cells, α -methyl-D-mannopyranoside was used at a concentration of 0.1 M. Acetone-fixed specimens were mounted in buffered glycerol and observed under epi-illumination with a Zeiss fluorescence microscope (aus Jena), equipped with a BG38 excitation filter

TABLE I
Genotypes of Yeast Strains

Strain	Genotype*
X2180	a / α <i>gal2/gal2</i>
X2180-1A	a <i>gal2</i>
X2180-1B	α <i>gal2</i>
XJ24-17a	a <i>ade6 lys2 arg4-17</i>
XY505-18C	α <i>his5-2 lys1-1 ura4-1</i>
XT1172-S245c	α <i>ade6 his6 leu1 met1 trp5-1 gal2 can1</i>
VC2	α <i>ste1-2</i> (class 1) same genotype as XT1172-S245c
VC3	α <i>ste3-3</i> (class 4) "
VC5	α <i>ste</i> ⁻ (class 14) "
VC8	α <i>ste</i> ⁻ (class 8) "
VC9	α <i>ste</i> ⁻ (class 6) "
VC73	α <i>ste</i> ⁻ (class 10) "
VN33	α <i>ste1-5</i> (class 2) "
VQ3	α <i>ste3-1</i> (class 4) "
XT1177-S47c	a <i>ade2-1 his2 lys1-1 trp5-18 gal2 can1</i>
VU3	a <i>ste</i> ⁻ (class 13) same genotype as XT1177-S47c
VW9	a <i>ste</i> ⁻ (class 7) "
VY3	a <i>ste</i> ⁻ (class 11) "
VY5	a <i>ste2-2</i> (class 3) "
VZ4	a <i>ste4(ts)</i> (class 5) "
VAB1	a <i>ste</i> ⁻ (class 5) "
VAB2	a <i>ste2-1</i> (class 3) "
VAC1	a <i>ste5(ts)</i> (class 5) "

* Gene symbols indicate mutations leading to requirements for the following: *ade* (adenine), *arg* (arginine), *his* (histidine), *leu* (leucine), *lys* (lysine), *met* (methionine), *trp* (tryptophan), *ura* (uracil). Additional symbols include the following: **a** or α (mating type), *can* (resistance to canavanine), *gal* (inability to ferment galactose), *ste* (sterile or nonmating); *ste*⁻ indicates a sterile mutation that has not yet been classified according to gene number.

and an OG1 barrier filter. Photographic records were made with Kodak Plus-X pan film.

RESULTS

Exponentially growing haploid *S. cerevisiae* cells were stained with FITC-Con A and examined by fluorescence microscopy. Each **a** cell or α cell, whether budded or not, exhibited uniform fluorescence. The data are not presented because a similar result has been reported previously (27). However, if cells of opposite mating type were incubated together without exogenous mating hormones until shmoos and zygotes appeared, altered staining patterns were observed. The zygotes (Fig. 1 A-E) stained nonuniformly; the conjugation bridge connecting the mating cells fluoresced more intensely than either of the two haploid parents or the diploid bud of the zygote. In the mating mixture, those cells which were not morphologically altered fluoresced uniformly with the intensity characteristic of the haploid parental portions of zygotes and the diploid buds in Fig. 1 or of untreated vegetative cells. The shmoos in

the population exhibited nonuniform fluorescence, with the wall of the growing end stained to a greater degree than the original wall that surrounded the cell. It is likely that the binding of FITC-Con A to the cells and zygotes in this experiment resulted from a specific saccharide-lectin interaction rather than a hydrophobic adhesion because staining was strongly inhibited in the presence of 0.1 M α -methyl-D-mannopyranoside. (Photomicrographs showing inhibitory effects of the glycoside on the staining of yeast cells by FITC-Con A have been published elsewhere [27].) These findings suggest that both the formation of shmoos and the development of zygotes are accompanied by the synthesis of altered cell surface areas.

Changes in cell wall ultrastructure brought about by the action of α -factor on **a** cells have been documented by Lipke et al. (14). Could the synthesis of the modified regions in the cell surfaces of shmoos that we have observed by fluorescence staining also be mediated by mating hormones? To investigate this possibility, we treated

haploid *S. cerevisiae* cells with the appropriate hormone and then with FITC-Con A. When the staining immediately followed the addition of hormone, the cells fluoresced uniformly (a cell, Fig. 2A; α cell, Fig. 3A). However, if the cells were incubated with the hormone for 2 h before staining, the shmoo that formed stained asymmetrically. The portion of the shmoo corresponding to

the original vegetative cell stained with approximately the same intensity as the cells exposed to FITC-Con A directly after the addition of hormone, whereas the newly synthesized region of the wall fluoresced more intensely (a cells treated with α -factor, Fig. 2B-D; α cells incubated with a-factor, Fig. 3B-D). The difference in fluorescence between the original and newly synthesized

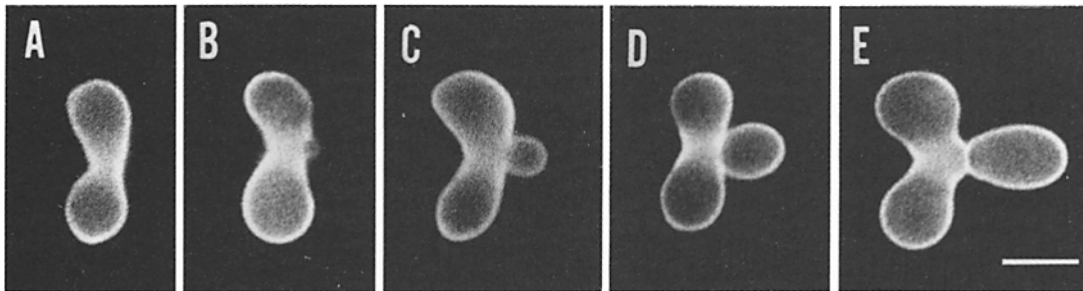


FIGURE 1 Zygotes of *S. cerevisiae* stained with FITC-Con A. Conjugation between α cells (X2180-1B) and a cells (X2180-1A) was allowed to proceed for 4 h before the mating mixture was treated with FITC-Con A as described in Materials and Methods. The examples shown represent zygotes in various stages of development that were present in the mixture. The fluorescence was greatest in the wall of the conjugation bridge both before bud initiation (A) and at all stages of bud development (B-E). The bar in this and subsequent figures represents 5 μ m.

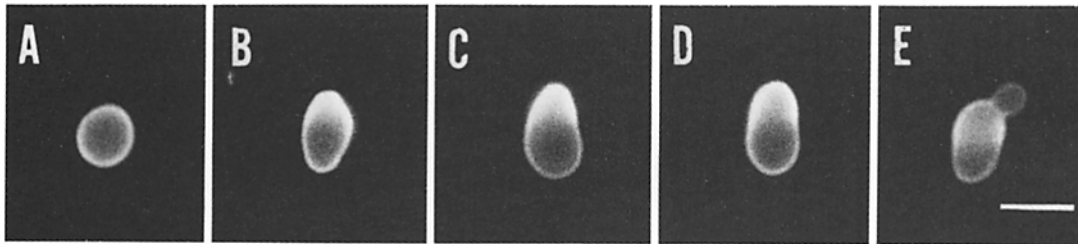


FIGURE 2 Fluorescence of a cells (X2180-1A) exposed to α -factor. Cell populations were stained with FITC-Con A immediately after the addition of α -factor (A) or after incubation with α -factor for 2 h (B-D) or 4 h (E); representative examples are shown.

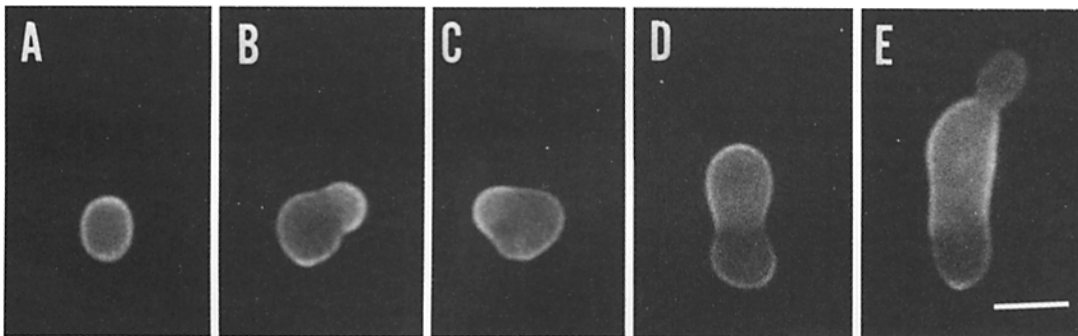


FIGURE 3 Fluorescence of α cells (X2180-1B) treated with a-factor. Cell populations were stained with FITC-Con A immediately after the addition of a-factor (A) or after incubation with a-factor for 2 h (B-D) or 4 h (E); representative examples are shown.

portions of the cell wall was consistently greater with **a** cells (compare Fig. 2 and 3). As in the previous experiment, staining was strongly inhibited by 0.1 M α -methyl-D-mannopyranoside. Thus, the shmoos which develop in cultures of haploid cells treated with the mating factors, like those seen in mating mixtures, differ from vegetative cells with respect to surface architecture.

The region of the shmoo that binds more FITC-Con A could arise either by an asymmetric rearrangement of existing wall material or by localized synthesis of a modified cell wall. To distinguish between these possibilities, we stained exponentially growing **a** and α cells with FITC-Con A, washed them with 0.15 M NaCl, and then incubated these with α -factor and **a**-factor, respectively, for 3 h. The shmoos developed at a normal frequency and were unstained at one end and dimly stained like vegetative cells at the other (data not shown). Rearrangement of existing wall material would have resulted in a migration of FITC-Con A into the elongated portion. Rather than this, the elongated portion was nonfluorescent, indicating the deposition of new surface polymers. Taken with the results of our previous experiments, this observation suggests that the mating hormones specifically induce *de novo* synthesis of new wall material that is either more reactive with or more accessible to FITC-Con A.

With the hormone concentrations employed (~2–4 U/ml), the shmoos generally began to overcome G1 arrest after 3 or 4 h and subsequently developed buds. When such cells were stained with FITC-Con A, the buds fluoresced with the intensity of vegetative cells, even though they always emerged from the more highly stained portion of the wall (**a** cells, Fig. 2E; α cells, Fig. 3E). Apparently, the influence exerted by the mating hormones upon cell wall synthesis is lost at the same time that the cells recover from G1 arrest. One explanation for the invariant location of the first bud could be the position of the nucleus (4, 19); when shmoos were exposed to both mithramycin to stain nuclei (23) and FITC-Con A, most of the nuclei were found in the portions of the cells that bound more Con A.²

The ability of a haploid cell to respond morphologically to a mating hormone is strictly dependent on its mating type; only **a** cells are sensitive to α -factor and conversely only α cells are affected by

a-factor. The cell wall alterations induced by the mating hormones are similarly specific for mating type, because asymmetric staining was not observed upon incubation of **a** cells with **a**-factor, α cells with α -factor, or **a**/ α diploids with either factor.

In the preceding experiments, we used strains X2180-1A (**a**) and X2180-1B (α), which are the standard wild-type strains from the Yeast Genetics Stock Center. All other **a** and α strains that have been studied also undergo morphological alterations in response to the appropriate mating hormone, unless they are nonmating mutants³ (2, 15). To determine if the asymmetric fluorescence patterns are unique to X2180-1A and X2180-1B or if they reflect a common response, we have treated the other strains listed in Table I with the appropriate factor and then stained them with FITC-Con A. The four **a** and α strains with wild-type mating ability formed shmoos that exhibited asymmetric fluorescence; in **a** cells, the staining asymmetry was always more pronounced than in α cells. In contrast, all the nonmating (*ste*) mutants (except VW9 and VC73; see below) did not undergo morphological changes and stained uniformly. The sterile strains in Table I are representative of at least five independent genes and nearly all of the 14 classes of mutants originally described by MacKay and Manney (16). Of particular significance are the temperature-sensitive mutants, VZ4 and VAC1, which can mate, produce **a**-factor, and respond morphologically to α -factor at 22°C, but lose these abilities at 34°C. At the higher temperature, these mutants, like the other sterile strains, stain uniformly after incubation with α -factor, but at 22°C, they exhibit the asymmetric fluorescence characteristic of normal mating strains.

The inability of the nonmating α mutants to respond morphologically to **a**-factor was originally determined microscopically after placing the α cells on an agar medium close to a heavy growth of **a** cells; the assay of the **a** mutants was conducted in a reciprocal manner (15). Although shmoos were not detected with VW9 (**a** *ste*) under those conditions, we observed a low percentage (10–20%) of elongated cells among the VW9 cells that had been incubated in liquid culture with α -factor. These altered cells exhibited the asymmetric fluorescence characteristic of **a** shmoos. When shmoos from unstained samples were isolated by

² J. Strazdis and V. L. MacKay. Unpublished observations.

³ V. L. MacKay. Unpublished observation.

micromanipulation and cultured on agar medium, the clones obtained still exhibited the nonmating phenotype and the low frequency of response to α -factor demonstrating that the VW9 shmoos are not revertants of the *ste* mutation. Moreover, the mutant still retains its mating-type specificity, because it was not affected by \mathbf{a} -factor. This was not the case with another nonmating mutant (VC73, α *ste*) in which ~30–35% of the cells even in vegetative cultures have the morphology and staining pattern of α shmoos (Fig. 4; compare to Fig. 3). Incubation of VC73 cells with either \mathbf{a} -factor or α -factor did not increase the frequency of shmoo-like cells. This “self-shmooing” phenotype might result from a regulatory defect, as suggested by the observations that the mutation in VC73 maps at the mating-type locus (24) and that this mutant exhibits at least one \mathbf{a} -specific trait, i.e., the “barrier” effect as described by Hicks and Herskowitz (13). At present, however, we have no additional information to indicate that the aberrant VC73 cells are the same as shmoos.

DISCUSSION

Clearly, some of the biochemical events of the mating process involve the cell surface which is the site of the primary physical interaction between mating cells (14, 19). Using fluorescently labeled concanavalin A, we have demonstrated localized surface alterations in zygotes leading to increased binding of the stain at the conjugation bridges between haploid parents. Differential binding of FITC-Con A was also seen with the unconjugated shmoos found in mating mixtures and in shmoos formed by the addition of the appropriate mating hormone to cultures of haploid cells but was not observed with vegetative cells. In a shmoo, the most intense fluorescence occurred at the growing end. From these findings it appears likely that, as a prelude to conjugation,

a haploid cell under the influence of a diffusible mating hormone synthesizes a region of its surface that differs from the vegetative wall. The modified cell wall areas are the sites at which two cells of opposite mating type initially bind to each other for the production of the conjugation bridge, possibly through the interaction of mating-specific agglutination factors that could be localized at these sites. The synthesis or activity of the agglutination factors appears to be induced by the mating hormones (1, 10, 30), although some strains seem to be somewhat constitutively agglutinative (30).

It was shown previously (26) that FITC-Con A stains yeast cells by interacting specifically with mannan, one of the three saccharide polymers of the cell wall. The localized increase in lectin binding observed in the experiments reported here could reflect a higher content of mannan, a change in mannan structure resulting in a greater number of nonreducing termini per molecule or an increased accessibility of the mannan. Lipke et al. (14) have compared walls of \mathbf{a} shmoos produced by treating \mathbf{a} cells with α -factor and of α cells incubated without α -factor. Their data indicate that \mathbf{a} shmoos have a relatively lower content of mannan and a higher proportion of glucan. Furthermore, the mannan polysaccharide in the shmoos has fewer mannotriose side chains and a greater number of unsubstituted mannosyl residues in the backbone, i.e., has a lower number of nonreducing termini. It should be noted that asynchronously growing \mathbf{a} cells were used in place of G1-arrested cells as the basis for this comparison; nevertheless, it seems likely that the decrease they observed in the mannan content of treated cells is not a general consequence of cell cycle events but is the result of hormone action, because bulk mannan is synthesized throughout the entire division cycle (22). From these considerations, we feel that our observations with FITC-Con A, at

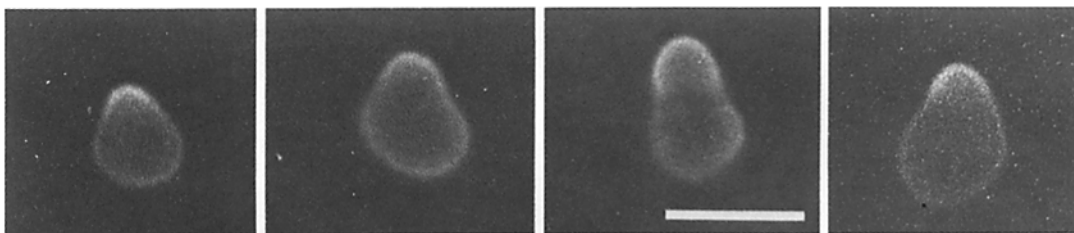


FIGURE 4 Shmoo-like cells in cultures of VC73. Cell populations of VC73 (α *ste*) were stained with FITC-Con A after incubation for 2 h without mating hormones, with \mathbf{a} -factor, or with α -factor. All three samples had the same frequency of aberrant cells and representative samples of these are shown.

least in the case of **a** cells, probably reflect an increased accessibility of surface mannan in the wall at the growing end of the shmoo. Lipke et al. (14) have found that **a** shmoos are more susceptible than **a** cells to lysis by glucanase which may indicate that the glucan in these shmoos is similarly more accessible. However, these authors did not observe a difference in sensitivity to exo- α -mannanase action when **a** shmoos and **a** cells were compared. With regard to α cells treated with **a**-factor, there are no data available which would permit us to decide whether increased binding of FITC-Con A by α shmoos is similarly due to increased accessibility of surface mannan.

One possible objection to the interpretations of our experiments and to the conclusions of Lipke et al. (14) has been raised by Manney and Meade (17); in both studies, only partially purified preparations of the mating hormones were used. Thus, the observed effects on the **a** cell surface could result from the action of another hormone in the preparation in addition to the characterized α -factor. If a second hormonal activity were responsible for the induction of cell surface changes, our results indicate that it must be mating-type specific. The supposition would also require that all the mutants we tested which cannot form shmoos in response to α -factor are also deficient in the ability to respond to the putative second hormone. Inasmuch as these mutants appear to carry only single mutations (16), it seems most probable that only one hormone is responsible for both the morphological changes and the cell surface alterations. It is noteworthy that the chemical synthesis of α -factor has been achieved (6), but it is not yet known whether the product, in addition to bringing about agglutinability, G1 arrest, and morphological changes in **a** cells, can also induce surface changes.

The evidence indicating that hormones are necessary for mating is still indirect and circumstantial (17). To date, the most compelling arguments linking the hormones with the mating process in *S. cerevisiae* derive from (a) the striking similarities between the responses elicited in haploid cells by purified hormone preparations and some of the events observed in mating mixtures (i.e., G1 arrest, morphological alterations, and induction of agglutinability [3, 5, 11, 21]), (b) the finding that the majority of either **a** or α mutants which were selected as nonmaters have simultaneously lost the ability to produce their respective mating hormone and to respond to the hormone of the

opposite mating type (15), and (c) the observation that all **a** mutants that have been isolated for resistance to α -factor are nonmaters (18). Although the experiments described here do not unequivocally demonstrate that the factors are required for mating, the fact that each zygote we observed exhibited increased binding of FITC-Con A in the wall of its conjugation bridge constitutes additional evidence that diffusible mating hormones and the cell surface changes they evoke are an integral part of the process that leads to cell fusion under normal laboratory conditions.

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