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Cell-Cell Recognition in *Saccharomyces cerevisiae*: Regulation of Mating-Specific Adhesion

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Mating-specific adhesion between haploid yeast cells of opposite mating type (**a** and α) was studied by using a quantitative agar plate assay. Washed **a** and α cells that had not previously been exposed to their respective opposite mating type ("naive" cells) adhered relatively weakly. In water, only 5 to 10% of the **a** cells stuck tightly enough to α cells to give rise subsequently to diploid clones on the assay plates. Under optimum conditions (pH 6 to 7, at least 0.1 M NaCl or 0.01 M Mg^{2+}), there was about 20% adhesion. Nevertheless, this weak binding defined a mating type-specific interaction because, even under optimum conditions, the homologous interactions (**a** with **a** and α with α) yielded only 3 to 5% cohesion. In contrast to these results, washed cells that had been preincubated in the cell-free culture medium of their opposite mating type ("preconditioned" cells) adhered quite strongly. The degree of adhesion between preconditioned cells (40 to 50%) was essentially unaffected by extremes of ionic strength, pH, and temperature and by the absence of divalent cation. This strong interaction was also mating type specific since cohesion between preconditioned cells of like mating type was only about 5%. The increase in agglutinability was obtained if only the **a** cells were preconditioned and could be induced by highly purified preparations of natural or synthetically prepared α-factor, an oligopeptide pheromone released by the α cells. The appearance of increased adhesiveness was blocked by an inhibitor of RNA synthesis and by an inhibitor of protein synthesis, but not by an inhibitor of polysaccharide synthesis. Adhesion between preconditioned cells could be inhibited by pretreatment with functionally univalent succinylated concanavalin A or with extracts from preconditioned cells of the opposite mating type. These results confirm in a quantitative manner that the recognition between conjugating cells of *S. cerevisiae* is a developmentally regulated event that is under the control of the mating type locus.

The mating response is a developmental alternative open to haploid cells of the yeast *Saccharomyces cerevisiae*. Mating is characterized by agglutination of the two haploid cell types, called **a** and α, followed by cell and nuclear fusion to form a diploid (8). Because it represents a process involving just two cell types, yeast conjugation is an attractive system for investigating the intercellular interactions that control and coordinate eucaryotic cell development. Of particular interest for understanding cell-cell recognition are the cell contacts that form during the adhesion step of mating.

Within 30 to 60 min after liquid cultures are mixed, **a** and α cells are agglutinated pairwise and in larger aggregates (20). During the early stages of conjugation, vigorous mechanical agitation is sufficient to separate these mating pairs. Thus, study of mating-specific adhesion in *S. cerevisiae* requires a gentle and sensitive assay method. Although there have been several recent studies of agglutination in this yeast, most notably by Yanagishima and his collaborators (27), the assays employed have been only semiquantitative or rather subjective and irreproducible (for a discussion, see D. N. Radin, Ph.D. thesis, University of California, Berkeley, 1976). Furthermore, in these other studies adhesion was measured by using boiled cells. It has been shown in another yeast species, *Hansenula wingei,* that a heat-labile mannoprotein is involved in the agglutination of one mating type, 21 cells, with its opposite mating type, 5 cells (6). Therefore, we felt that a quantitative and physiologically relevant technique was essential for detecting possible changes in mating-specific adhesiveness and for identifying and purifying the cell surface components that mediate cell-cell recognition during conjugation. In this report, we describe an extension of the procedure for measuring cell aggregation originally devised by Campbell (4) and its use for determining the

effects of various conditions and treatments on the adhesion between **a** and α cells. We demonstrate that the ability of yeast cells to agglutinate is specifically stimulated before conjugation and is apparently under the pheromonal control of α-factor, an oligopeptide produced by the α haploid cells (25).

MATERIALS AND METHODS

Materials. Chemicals were obtained from the following sources: cycloheximide, methyl-α-D-mannoside, and ethyleneglycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) from Sigma Chemical Co.; (ethylenedinitrilo)tetraacetic acid (EDTA) from Mallinckrodt; D-mannose and melibiose from Pfanstiehl; mannose-1-phosphate from Boehringer Mannheim Corp.; glucose-1-phosphate and 2-deoxy-D-glucose from Calbiochem; concanavalin A (ConA) from Pharmacia Fine Chemicals, Inc.; and media constituents from Difco Laboratories. All other chemicals were reagent grade. Lomofungin was the gift of G. B. Whitfield, Jr., The Upjohn Co. Functionally univalent succinylated ConA was prepared by the method of Gunther et al. (9).

Organisms and growth conditions. The genotypes and origins of the strains used are given in Table 1. The haploids employed in this work were selected because they all exhibited a low degree of nonspecific self-aggregation ("clumpiness") such that the cells grew essentially monodisperse in liquid culture. Cells were grown in YPD, a complex medium, or in SD, a minimal medium, whose compositions have been described previously (21). For routine cultivation of auxotrophs, SD was supplemented with 20 to 200 μg of the required nutrients per ml. All solid media contained 2% Difco agar, except top agar (0.7%).

Adhesion assay. Small cultures of YPD (25 ml) were inoculated to a cell density of about 10^6/ml with fresh single-colony isolates and were grown with vigorous aeration at 30°C to a cell density of about 2×10^8/ml (approximately 320 units on a Klett-Summerson photoelectric colorimeter with a no. 66 red filter), which corresponds to the late exponential-early stationary phase of growth. Cells were then harvested and washed in sterile water or in an appropriate sterile nonnutritive buffer by three brief (5-min) cycles of centrifugation and resuspension. This thorough washing insured that cells were essentially metabolically inactive during the assay. After determination of cell number in a Petroff-Hausser counting chamber, the final suspensions were adjusted to 2×10^7 cells per ml. When necessary, cells were maintained on ice before an experiment. To initiate an assay, equal volumes (0.5 ml) of the two different haploid cell suspensions to be tested were mixed thoroughly in small screw-capped test tubes (13 by 100 mm). The mixtures were immediately sedimented at room temperature by centrifugation for 5 min in a swinging bucket rotor of a Sorvall GLC-1 centrifuge at 1,000 rpm. The cell pellets were then incubated for 30 min in a 30°C water bath (although incubation times as short as 5 min gave similar results), resuspended by gentle hand rocking, diluted, and plated on assay medium by the soft agar overlay method to yield approximately 250 colonies ($Z + H$; see below) per petri dish. The resuspended cells were then subjected to vigorous Vortex mixing and plated on media selective for each haploid parent (h_1 and h_2; see below). Plates were incubated at 30°C and scored after 3 to 4 days. In all experiments, care was taken to manipulate and pipette the resuspended adhesion mixtures slowly and gently to minimize shear forces. Replicate platings performed in parallel were reproducible with respect to the recovery of diploid clones, suggesting that the handling procedure did not destroy cell aggregates. All values reported represent the average of at least two separate determinations, each done in triplicate.

TABLE 1. *S. cerevisiae strains used*

Strain	Genotype[a]	Source
X2180-1A	**a** *SUC2 mal gal2 CUP1*	R. K. Mortimer
X2180-1B	α *SUC2 mal gal2 CUP1*	R. K. Mortimer
X2180-G2C	**a** *SUC2 mal gal2 CUP1*[b]	V. L. MacKay
S1795A	**a** *ade6 his4 trp5 ura1 gal2*	R. K. Mortimer
S1799D	α *ade6 his4 trp5 gal2*	R. K. Mortimer
S2684D	**a** *ade1 his2 trp5 arg7 his8 leu1*	R. K. Mortimer
A364A	**a** *ade1 ade2 ura1 his7 lys2 tyr1 gal1*	L. H. Hartwell
D160-4D	**a** *ade1 his1 trp2 hom3 ilv1 met1 arg6 ura3*	R. K. Mortimer
D160-2C	α *ade1 his1 trp2 hom3 ilv1 met1 arg6 ura3*	R. K. Mortimer
1453-3A	**a** *suc MAL2 MEL1 his4 leu2 ser*	R. K. Mortimer
AN33	α *thr1 arg1*	R. K. Mortimer

[a] Genetic nomenclature for *S. cerevisiae* is according to Plischke et al. (17).

[b] This **a** cell derivative is purported to produce a high level of an activity (2) that arrests cell division and induces the morphological elongation of α cells (V. L. MacKay, personal communication).

Preparation of preconditioned cells. Preconditioned cells were prepared by suspending freshly grown cells at a concentration of 1×10^8 to 2×10^8/ml in cell-free conditioned medium from cultures of the opposite mating type and incubating at 30°C with aeration for an appropriate period of time, usually 1 h. Such preconditioned cells were then washed thoroughly, as described above, and used in the adhesion assay. Conditioned media were prepared before each experiment by growing X2180-G2C (**a**) and X2180-1B (α) for 48 h in SD, followed by removal of the cells by centrifugation for 45 min at 12,000 rpm in an SS-34 rotor of a Sorvall RC-2B centrifuge at 4°C. Conditioned media were diluted with 1/10 volume of fresh YPD just before use. Preconditioned **a** haploids were also prepared by the addition of highly purified natural α-factor or chemically synthesized α-factor (5) directly to the culture medium at a final concentration of 5 to 10 units per ml at 1 h before harvesting the cells. One unit of α-factor activity was defined as the amount of peptide sufficient to produce a pronounced morphological elongation in at least 20% of the α cells in the microtiter assay system described previously (5).

Other methods. Extracts of washed preconditioned cells (2 to 4 g, wet weight) were prepared by suspending them in 2 volumes of lysis buffer [1 mM

phenylmethylsulfonyl fluoride-5 mM EDTA-30 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.0)] and either rupturing by two passages through a chilled French pressure cell at 20,000 lb/in^2 or autoclaving for 3 min at 120°C (10). Extracts were clarified by centrifugation at 17,000 × *g* for 30 min at 4°C, and the supernatant fluids were used immediately thereafter. In the preparation of such extracts, precautions were taken to use sterile implements, solutions, and containers. Protein concentration was determined by the technique of Lowry et al., using bovine serum albumin as a standard (14). Carbohydrate content was determined by the phenol-sulfuric acid method of Dubois et al., using mannose as a standard (7).

RESULTS

Cell-cell adhesion is measured in the cosedimentation assay. If an equal number of thoroughly washed **a** and α cells were mixed in nonnutritive buffer and cosedimented, upon plating out the gently resuspended cell pellet by the soft agar technique onto a medium that permitted growth and therefore completion of the mating process, diploid clones arose. If the **a** and α cells carried complementing auxotrophic markers and the medium contained only limiting concentrations of the required nutrients, then the prototrophic diploid clones could be clearly distinguished by their large size from colonies of either haploid (Fig. 1). The following control experiments indicated that the number of diploid clones was a direct and quantitative measure of the degree of adhesion between the haploids. First, no diploid colonies appeared if the **a** cells and the α cells were separately sedimented and resuspended and then mixed together in the soft agar and plated. This result showed that the diploid clones did not arise from chance congruence of haploid colonies on the indicator plates. Second, and most importantly, if the **a** and α cells were cosedimented, but resuspended after incubation by brief (5-s) Vortex mixing, no diploid clones were found, whereas there was no reduction in the number of haploids recovered. Thus, diploid colonies appeared to represent those **a**-α cell pairs that adhered to each other tightly enough, as the result of their intimate contact in the pellet, to survive a gentle resuspension such that they could fuse when growth resumed. In other words, the method employed seemed to separate operationally the adhesion between cells of opposite mating type from subsequent steps in the conjugation process. The results of such experiments were expressed as percent adhesion for the **a** haploid, which was calculated as $100 \times Z\ (h_1 + h_2/2Z + H)/h_1$, where Z = the number of diploid clones on the assay plate, H = the number of haploid clones on the assay plate, h_2 = the number of α cells on the selective plate, and h_1 = the number of **a** cells on the selective plate. This formula corrected for any non-uniformity in the solution of a gently resuspended pellet. It should be noted that this treatment also assumed that the aggregates that ultimately formed a diploid contained an average of one **a** cell and one α cell. That this was indeed the case, when the ratio of input cells was near one, was demonstrated by the fact that the difference between the number of haploid cells in the original mixture and the number of free haploids recovered on the indicator plates was essentially accounted for by twice the number of diploid clones. However, in matings of preconditioned cells, the recovery of haploid clones on the assay plates was often somewhat reduced, suggesting that the aggregates were somewhat larger (three to five cells).

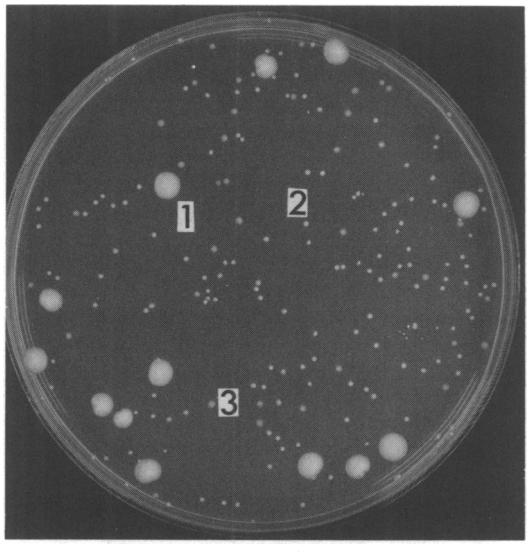

FIG. 1. *Plate assay of mating-specific adhesion. Washed cells of AN33 and A364A were mixed, cosedimented, diluted, and plated as described in the text onto glucose-minimal salts medium (SD) containing limiting concentrations of the nutrients required by these strains. 1, Prototrophic diploid colony; 2, auxotrophic haploid* **a** *parent colony; and 3, auxotrophic haploid* α *parent colony.*

We have used this method to explore the effects of various conditions and treatments on adhesion between viable cells of opposite mating type to define in a physiologically significant way the properties of the cell surface molecules responsible for this interaction.

Adhesion between yeast cells is mating type specific. The agglutination between "naive" cells, which had not previously been exposed to their opposite mating type, was examined first. Washed naive **a** and α cells showed a low degree of adhesion. Their interaction was

greatly affected by the composition of the buffer in which the adhesion was measured. In water, adhesion between **a** and α cells was always less than 10%. Because raising the ionic strength of the milieu permitted considerably more adhesion (Fig. 2), the low value observed in water was presumably the result of charge repulsion between cells which are net negatively charged due to the outermost cell wall polymer, phosphomannan (1). The presence of salt could be completely substituted for by lower concentrations of Mg^{2+} and Ca^{2+}, although neither divalent cation seemed essential for the interaction (Table 2). Other divalent metal ions of interest (Zn^{2+} and Cu^{2+}) were tested, but were found to have a drastic effect on cell viability, as has been observed by others. That the interaction between naive cells depended on the state of ionizable groups on the cell surface was also supported by the response of adhesion to changes in pH. As shown in Fig. 3, a somewhat pronounced optimum for adhesion was found between pH 6 and 7 in four separate buffering systems. The binding between naive **a** and α cells was not reduced by the presence of phosphate or various monosaccharide and monosaccharide-phosphate compounds (1 to 10 mM). Under optimum conditions (pH 6 to 7, 0.1 M NaCl or 0.01 M Mg^{2+}), only about 20% adhesion was ever observed between naive cells of opposite mating type. Nevertheless, this binding appeared to define a mating type-specific interaction because, even under optimum conditions, homologous combinations, **a** with **a** (A364A + D160-4D, A364A + 1453-3A, A364A + S2684D, and S2684D + S1795A) and α with α (AN33 + S1799D and AN33 + D160-2C), yielded only 3 to 5% cohesion (as scored by replica plating all colonies onto selective media to detect mixed clones).

Mating type-specific adhesion is increased before conjugation. In contrast to the results for naive cells, **a** and α cells that had

TABLE 2. *Effect of divalent cations on adhesion of naive cells*

Metal ion	Adhesion[a] (%)	
	Ion alone	Ion + NaCl
Mg^{2+}	26	20
Ca^{2+}	17	23
Mn^{2+}	10	18
Co^{2+}	9	21
Fe^{2+}	8	14
None	2	21

[a] Cells (A364A and AN33) were washed three times with 10 mM EDTA-10 mM EGTA (pH 7.0) and then three times with 10 mM solutions of the divalent cations to be tested, either lacking or containing 100 mM salt, and subjected to the adhesion assay as described in the text. Dilution tubes and soft agar contained the same ions at the same final concentrations.

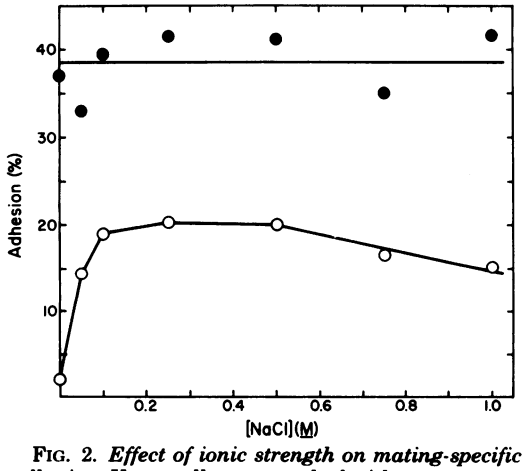

FIG. 2. *Effect of ionic strength on mating-specific adhesion. Yeast cells were washed with water, resuspended in NaCl solutions at the indicated concentrations, and used in the adhesion assay, as described in the text. Symbols: ○, naive cells; ●, preconditioned cells.*

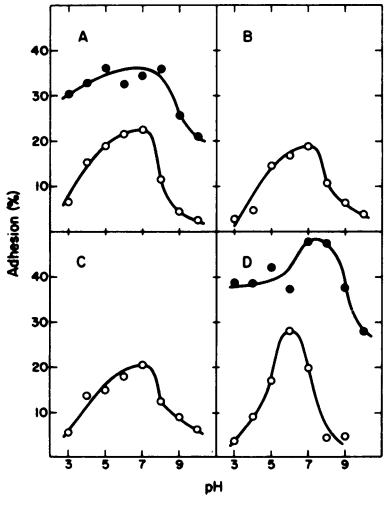

FIG. 3. *Effect of pH on mating-specific adhesion. Yeast cells were washed with water, resuspended in either 25 mM succinate-25 mM Tris (A) or 25 mM citrate-25 mM Tris (B) or 25 mM potassium phosphate-25 mM succinate (C) or 25 mM potassium phosphate-25 mM citrate (D) that had been adjusted to the indicated pH values with either HCl or NaOH, and then used in the adhesion assay as described in the text. Symbols: ○, naive cells; ●, preconditioned cells.*

been preincubated in the cell-free culture medium of their respective opposite mating type ("preconditioned" cells) showed markedly increased agglutinability. As shown in Fig. 2, the degree of adhesion between preconditioned cells was 30 to 50%, even in water. This interaction could be further distinguished from that between naive cells in that it was much less affected by extremes of pH (Fig. 3). Unlike naive cells, the adhesion between preconditioned cells in water was neither enhanced nor reduced by the presence of divalent cations. This finding distinguished mating-specific adhesion of preconditioned cells from the Ca^{2+}-dependent "flocculation" of some *S. cerevisiae* strains (11). Adhesion between preconditioned cells was essentially unaffected by low temperature (4°C) or by the addition of phosphate or monosaccharide or monosaccharide-phosphate compounds (1 to 10 mM). Again, no diploids appeared on the assay plates if cell pellets were subjected to vigorous Vortex mixing just before plating. Thus, the observed increase in adhesion between preconditioned **a** and α cells did not represent cell pairs that were able to fuse during the assay procedure before plating. The strong interaction between preconditioned cells also appeared to be mating type specific, since cohesion between preconditioned cells of like mating type, **a** with **a** (A364A + S2684D and S2684D + S1795A) and α with α (AN33 + S1799D), was only 5% or less.

Increased adhesion is induced by α cell pheromone. As shown in Table 3, the increase in agglutinability between **a** and α cells was induced only by incubating the cultures in conditioned media of the opposite mating types. Little or no increase in adhesiveness was caused by incubating the cells in conditioned media produced by the same mating types. It was only necessary to precondition the **a** cells to observe the increased adhesion (Table 3). This suggested that the ability of these cells to agglutinate was directly induced by α-factor. Indeed, the same increase in adhesion was obtained if the **a** cell cultures were preincubated with highly purified natural α-factor or synthetic α-factor (Table 3). All of the **a** haploid strains tested (A364A, 1453-3A, S2684D, and S1795A) showed increase adhesiveness toward α cells after preconditioning (three- to eightfold), although A364A was used in essentially all subsequent experiments. Concentrations of α-factor that were too low to cause the pronounced morphological elongation ("schmoo" formation [15]) known to be elicited by the pheromone (12) were still effective at inducing the increased agglutinability of **a** cells (Table 4). In addition, the increase in adhesiveness did not appear to be correlated with any increase in the proportion of unbudded cells accumulated during the preincubation, as determined by microscopic examination.

TABLE 3. *Effect of preconditioning on adhesion of yeast cells*[a]

a Cells preconditioned with:	α Cells preconditioned with:		
	Nothing	**a** Cell supernatant	α Cell supernatant
Nothing	8[b]	8	
α Cell supernatant	28	39	42
Purified α-factor		37	
Synthetic α-factor		44	
a Cell supernatant			11

[a] After preconditioning as described in the text, under the indicated conditions, cells (A364A and AN33) were washed with sterile water and used in the adhesion assay.

[b] Percent adhesion.

Increased adhesion reflects cell surface changes that require macromolecular synthesis. To determine whether the induction of increased adhesiveness by α-factor required new macromolecular synthesis, the effects of specific inhibitors were investigated. Maximal enhancement of the adhesiveness of **a** cells was attained by 30 to 45 min after exposure to α-factor-containing medium (Fig. 4). As shown in Fig. 4A, this increase in agglutinability was completely blocked by the presence of the protein synthesis inhibitor cycloheximide during preconditioning of the **a** cells. A similar inhibition was observed in the presence of the antibiotic lomofungin, a Zn^{2+} chelater that inhibits mRNA and rRNA synthesis in yeast cells (16). Perhaps surpris-

TABLE 4. *Effect of α-factor concentration on stimulation of* **a** *cell adhesiveness*

α-Factor concn (units/ml)	Adhesion (%)[a]
0	6
0.001	18
0.01	21
0.05	25
0.1	29
0.5	25
1.0	35
3.0	34
10.0	45

[a] Washed cells of A364A were resuspended in SD containing the indicated concentrations of partially purified α-factor, incubated on a roller drum at 30°C for 1 h, washed with water, and then challenged in the adhesion assay with water-washed cells of AN33 that had been preconditioned in medium from a culture of X2180-G2C, as described in the text.

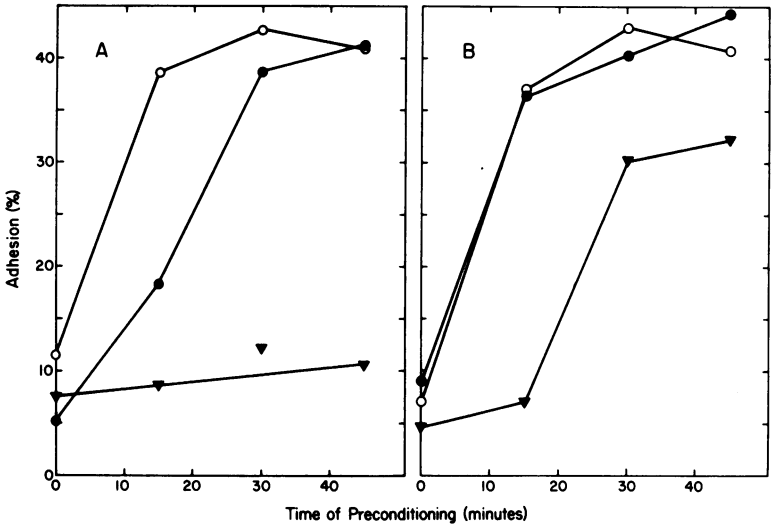

FIG. 4. *Effect of inhibitors of macromolecular synthesis on preconditioning. Washed cells of A364A were preconditioned at 30°C under the conditions described below for the indicated times, washed three times with water, and then challenged in the adhesion assay with water-washed cells of AN33 that had been preconditioned in a similar manner. Symbols:* ○, *preconditioned in the absence of any inhibitor;* ▼, *preconditioned in the presence of inhibitor; and* ●, *preconditioned in the absence of inhibitor, but inhibitor added after the indicated times. The inhibitors were cycloheximide (15 μg/ml) (A) and 2-deoxyglucose (500 μg/ml) (B).*

ingly, the increase in adhesiveness upon preconditioning was delayed somewhat but was not prevented by the presence of 2-deoxyglucose (Fig. 4B), an analog of both glucose and mannose that, at the concentration used here (500 μg/ml), is an effective inhibitor both of the **a** cell elongation induced by α-factor (13) and of the cell fusion step of conjugation (23).

If the requirement for protein synthesis in the enhancement of agglutinability represented the elaboration and release during preconditioning of diffusible extracellular lectin-like molecules, then pretreatment of naive cells with supernatant fluid from the culture medium of preconditioned cells might be expected to increase their adhesion. However, when naive cells were exposed to supernatant solutions from preconditioned cultures of the same, opposite, or both mating types, no increase in their agglutinability was observed, as long as cycloheximide was present to prevent preconditioning of the naive test cells themselves. This finding suggested that the macromolecular synthesis required for increased adhesiveness represented the insertion of new or the alteration of preexisting surface components that are tightly bound to the cells. Two experiments indicated that the components responsible for this cell-cell recognition are indeed integral surface components. First, whereas pretreatment of the cells with concentrations of ConA as high as 1 mg/ml did not block mating between preconditioned **a** and α cells, functionally univalent succinylated ConA at this concentration did effectively reduce agglutination (Fig. 5). (The residual adhesion of preconditioned cells observed at 1 mg of succinylated ConA per ml may represent the degree of adhesion that would be observed between naive cells at the ionic strength of the ConA buffer.) Second, pretreatment of either **a** or α preconditioned cells with crude extracts of preconditioned cells of the opposite mating type completely inhibited agglutination (Table 5). Interestingly, if both **a** and α cells were pretreated with extract, agglutination was not completely eliminated. This latter result also suggested that the extracts did not block agglutination between **a** and α cells through hydrolysis of (or other degradative action on) cell surface components. Extracts prepared by autoclaving concentrated suspensions of preconditioned cells were much less effective for inhibiting adhesion between preconditioned cells.

DISCUSSION

Using the cosedimentation assay, we have clearly demonstrated that prior exposure of **a** cells to α-factor markedly increases their adhesiveness toward α cells. Presumably, the value of such elevated agglutinability for the mating process is to enhance the frequency of *productive* collisions between cells of opposite mating type. Our finding that induction of increased adhesiveness required only 30 to 60 min (much less than one generation) suggests that the cell-cell recognition step in mating takes place before

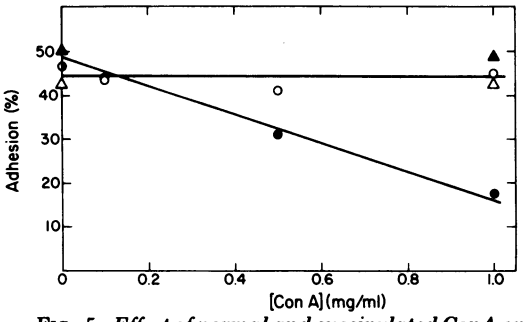

FIG. 5. *Effect of normal and succinylated ConA on adhesion of preconditioned cells. Washed preconditioned cells (A364A and AN33) were resuspended in buffer (0.1 M NaCl-25 mM succinate [pH 5.5]) containing either normal ConA (open symbols) or succinylated ConA (closed symbols) at the indicated concentrations, incubated for 5 min at 30°C, and then used in the adhesion assay, as described in the text. △, ▲, Methyl-α-D-mannoside (10 mM) present.*

TABLE 5. *Inhibition of adhesion by extracts of preconditioned cells*[a]

a Cells treated with:	α Cells treated with:		
	Nothing	a Cell French press extract[b]	a Cell autoclave extract[c]
Nothing	44[d]	0	25
α Cell French press extract[b]	0	14	
α Cell autoclave extract[c]	37		61

[a] Portions (0.1 ml) of suspensions (10^8/ml) of washed preconditioned cells (A364A and AN33) were diluted into 1 ml of the indicated freshly prepared extracts and incubated for 1 h at 30°C on a roller drum. The cells were then mixed with their opposite mating type for the adhesion assay, as described in the text.

[b] Protein, ~10.0 mg/ml; carbohydrate, ~3.0 mg/ml.

[c] Protein, ~0.5 mg/ml; carbohydrate, ~0.5 mg/ml.

[d] Percent adhesion.

the cell cycle arrest that occurs in the presence of α-factor (3). Indeed, we found that maximum agglutinability was achieved before there was any detectable increase in the proportion of unbudded cells or any change in the gross morphology of a cells. Taken together, these results define the stimulation of agglutinability as yet another biological function of the α pheromone.

The fact that agglutinability of yeast cells is increased during conjugation has also been reported recently by Yanagishima and his coworkers (27), using several different assay methods. These techniques include spectrophotometry (for example, comparison of the absorbance of a and α cell mixtures before and after sonic treatment) and direct microscopic inspection. Radin has recently made similar observations by following particle size with a Coulter Counter (Radin, Ph.D. thesis). However, the major drawback in all of these procedures is that they provide a relatively insensitive measurement of total cellular aggregation that is difficult to distinguish from nonspecific interactions such as flocculation. In contrast, the assay used in our study provides a direct quantitation of the contacts between a and α cells that lead to productive zygote formation. This is, therefore, a much more reliable index of interactions that are important in the conjugation process. For example, we could clearly demonstrate the increased agglutinability of preconditioned A364A cells, whereas Shimoda et al. have claimed that strain A364A is only weakly agglutinable (22).

Shimoda et al. have also stated that the enhanced agglutinability that they have observed during conjugation is induced in a cells by a peptide factor produced by α cells, which they have termed "α substance I" (24). Based on the chemical and physical properties of their purified material (18), these workers felt that the substance was different from α-factor, although this does not seem to be borne out in their subsequent work (19). As shown here, highly purified preparations of natural α-factor and chemically synthesized α-factor are able to induce the increased adhesiveness of a cells. The fact that synthetic α-factor fully stimulated agglutinability leaves little doubt that the pheromone is the sole primary signal for eliciting this biological response in a cells.

A high concentration of ConA, which has an affinity for α-D-mannopyranosyl groups such as those found in yeast mannan (26), did not block mating between preconditioned a and α cells. On the other hand, functionally univalent succinylated ConA was an effective inhibitor. Obviously, succinylated ConA binding masks or sterically blocks the normal cell surface agglutinins required for mating-specific adhesion. Because normal ConA must cover these same sites, it presumably can substitute in lieu of the normal agglutination process because of its multivalent nature. Similarly, pretreatment of both preconditioned haploids with extracts of preconditioned cells of opposite mating type did not eliminate their agglutination completely, whereas pretreatment of only one cell type did. Considering these observations, we favor the idea that the cell surface components that confer agglutinability are multivalent on both the a and α cells (Fig. 6). Extracts of preconditioned cells prepared by autoclaving, which is known to extract most of the cell wall manno-protein (1), were much less effective in inhibiting adhesion of preconditioned cells. However, it is possible

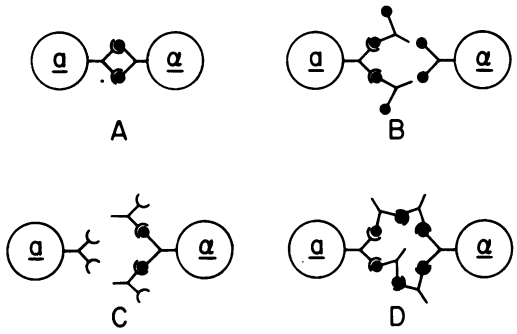

FIG. 6. *Model of mating-specific adhesion in S. cerevisiae. (A) Normal agglutination; (B) a cell pretreated with extract from preconditioned α cells, agglutination blocked; (C) α cell pretreated with extract from preconditioned a cells, agglutination blocked; and (D) both a and α cells pretreated with extracts of preconditioned cells of the opposite mating type; some agglutination can still occur due to the proposed multivalent nature of the cell surface agglutinins.*

that the agglutinins are heat labile or that our autoclave extracts were too dilute. Indeed, Yanagishima's laboratory has reported recently (10) that a heterogeneous glycoprotein fraction can be released from a cells by autoclaving. In contrast to our data, this material appeared to mask the agglutinability of α cells toward a cells in a univalent fashion. Unfortunately, the particular a strain used in their work (T7) shows an intrinsically high degree of agglutinability (27). A comparison with our data is further complicated by the fact that boiled cells were used in their assays and that the a cells were not exposed to α-factor-containing medium before extraction. Further fractionation of the inhibitory species in French pressure cell lysates of preconditioned cells may be the best way to identify and characterize the cell surface agglutinins responsible for mating-specific adhesion in *S. cerevisiae*.

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