Roles of sexual cell agglutination in yeast mass mating

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The \( \text{ag} \alpha_1 \) mutant \( \text{MAT} \alpha \) cells specifically lack the cell surface \( \alpha \)-type sexual agglutination substance, which is also called \( \alpha \)-agglutinin. Because the mutant cells (\( \text{MAT} \alpha \text{ag} \alpha_1 \)) cannot form aggregates with \( \text{MAT} \alpha \) cells, \( \text{MAT} \alpha \text{ag} \alpha_1 \) cells are unable to mate with \( \text{MAT} \alpha \) cells when they are co-inoculated in a liquid medium, and the mating is attenuated on solid medium. The attenuated mating ability shown in the previous studies gave us a vague idea about a physiological function of the sexual agglutinability. In order to solve the question, mating behavior of \( \text{MAT} \alpha \text{ag} \alpha_1 \) cells was investigated here under conditions where the contact between \( \text{MAT} \alpha \) and \( \text{MAT} \alpha \) cells is assisted by physical methods. A synthetic mutation \( \text{ag} \alpha_1::\text{URA3} \) was constructed and used as well as \( \text{ag} \alpha_1-1 \) for this study to ensure the genetic defect. When a mixture of \( \text{MAT} \alpha \) and \( \text{MAT} \alpha \) cells was kept on filter membrane placed on relatively dry agar medium, even \( \text{ag} \alpha_1::\text{URA3} \) mutant cells mated as efficiently as the wild type (\( \text{AG} \alpha_1 \)) cells did. On filter membrane placed on moist agar medium, \( \text{ag} \alpha_1 \) mutants mated 10-fold less efficiently than wild type cells did. The mutant cells mated 10000-time less efficiently than the wild type cells in a pellet formed by brief low speed centrifugation. In contrast, the wild type \( \text{MAT} \alpha \) cells mated well under all conditions tested. Under the pellet condition, a mixture of \( \text{MAT} \alpha \) and \( \text{MAT} \alpha \text{AG} \alpha_1 \) cells formed an extended and cotton-like pellet while a mixture of \( \text{MAT} \alpha \) and \( \text{MAT} \alpha \text{ag} \alpha_1 \) cells formed a compact and tight pellet. These results suggest that sexual cell agglutination contributes not only to cell contact between \( \text{MAT} \alpha \) and \( \text{MAT} \alpha \) cells thereby stabilizing \( \alpha-\alpha \) cell pairs, but also to construction of a uniquely organized ultra structure favorable for zygote formation and subsequent growth of diploid cells. The mating specific extended pellet formation was observed also in 4 pairs of \( \alpha \) and \( \alpha \) strains in ascosporogenous yeast genera \textit{Hansenula} and \textit{Pichia}.

Key words: prelude of cell fusion, sexual cell agglutination, sexual cell interaction, zygote formation

INTRODUCTION

Haploid cells of ascosporogenous yeasts exhibit either of two mating types. In the yeast \textit{Saccharomyces cerevisiae}, the mating type is determined by co-dominant regulatory genes \( \text{MAT} \alpha \) and \( \text{MAT} \alpha \) at the mating type locus \( \text{MAT} \) on chromosome III (Herskowitz and Oshima, 1981). When cells of the two mating types are mixed and co-cultured, they start mating processes to produce diploid \( \text{MAT} \alpha \text{MAT} \alpha \) cells. At initial steps to cell fusion, \( \text{MAT} \alpha \) and \( \text{MAT} \alpha \) cells communicate each other using mating type specific substances. Gametic differentiation is induced by signal transduction triggered by mating pheromones and their receptors, and the physical cell adhesion is assured by agglutination by cell surface glycoproteins (Yanagishima and Yoshida, 1981; Betz et al., 1981; Cross et al., 1988; Sprague, 1991). Those processes on the cell surface are important for mating because gametic inductions and cell adhesion are prerequisite for the subsequent fusion of the cell wall and plasma membrane in mating cell pairs. The sexual agglutination is brought about by complementary binding of the cell wall glycoproteins, \( \alpha \)- and \( \alpha \)-type agglutination substances (Yanagishima and Yoshida, 1981), which are also called agglutinins. A chromosomal gene \( \text{AG} \alpha_1 \) on the chromosome \( X \) is the structural gene for the \( \alpha \) type agglutination substance. Mutations in \( \text{AG} \alpha_1 \) gene cause \( \alpha \) cells defective in agglutinability while other sexual functions are normal in the mutants (Suzuki and Yanagishima, 1985, 1986; Lipke et al., 1989; Doi et al., 1989).

In liquid media, \( \text{MAT} \alpha \text{ag} \alpha_1 \) mutant cells are inevita-
bly unable to mate (Suzuki and Yanagishima, 1985; Lipke et al., 1989; Doi et al., 1989). On filter membrane, they show high mating ability, about one 5th of the wild type efficiency (Suzuki and Yanagishima, 1985). As pointed out by Cross et al. (1988), the high but significantly less mating efficiency of the mutant cells even under the filter membrane condition has put a question whether the sexual cell agglutination functions only as a paste to appose membrane condition has put a question whether the sexual cell agglutination functions only as a paste to appose cell agglutination and the sexual agglutination substances. It was found that filter membranes placed on relatively dry agar medium made the mutants capable to mate as efficiently as wild type cells. A slight difference of moisture of the filter membrane affected mating efficiency and zygote length when MATα cells had agα1 mutations. Apparently larger and softer pellets were formed after brief low speed centrifugation in mixtures of MATα and wild type MATα cells than in mixtures of MATα and MATα agα1 cells. Roles of sexual cell agglutination were discussed, based on results obtained here.

**GENE DISRUPTION.** A synthetic agα1 mutation was constructed and introduced into yeast genome as follows. A portion of the AGα1 gene (from base position 3830 to 4741 according to Lipke et al., 1989) was amplified by a PCR reaction using a primer set AGGGAGGGCGATGAATTC and GCCGTGTTCCTACTGTTGGA with total genomic DNA extracted from KS13-1D strain. The fragment was cloned in the HincII site of pUC19. In the unique NsiI site of the insert in the recombinant plasmid, the URA3 gene was inserted to interrupt the AGα1 ORF. The resultant plasmid pUC-agα1:URA3 was cut with BamHI before transformation of yeast strains DBY746 and KK4 to replace the chromosomal AGα1 gene with the synthetic mutant gene agα1::URA3 by the one step gene disruption method (Rothstein 1991). Introduction of the synthetic mutation on the locus was confirmed by PCR experiments using the same set of primers with total genomic DNAs from the transformants as well as Southern hybridization experiments. Recombinant DNA technologies including transformation were according to the standard ones (Maniatis et al., 1982; Suzuki et al., 1989; Oka et al., 1999).

**AGGLUTINATION SUBSTANCE PREPARATION.** For physiological experiments α-agglutination substance was prepared as follows. T56 (MATα) cells were collected from a culture at early stationary phase in YHG. The active fraction was extracted by the autoclave method (Yoshida et al., 1976) from 250 g wet weight cells and purified according to Hagiya et al. (1977) and Yoshida et al. (1976). Purified substance was dialyzed against water and lyophilized.

**HELPER CELL PREPARATION.** KS2-17A (MATα) cells were grown to early stationary phase in YHG. The cells were washed twice with 10 mM phosphate buffer pH 5.5. The washed cell suspension was boiled for 10 min and then chilled on ice. The boiled cells were washed twice with the above buffer. Thus treated cells retained high agglutinability as α cells, while their viability rate was less than 10⁻³. The cells were called helper cells.

**ZYGOTE FORMATION.** For mating reaction, 4 x 10⁶ MATα cells and the same number of MATα cells were mixed, then treated under the following conditions.

1. **Mating on filter membrane condition:** the cells were suspended in 10 ml YPD and then the cells were trapped on a disc of filter membrane (type SM11304, Sartorius GmbH, Göttingen) by sucking off the medium, using a filtration equipment which has a filtration area of 2.1 cm² (type KG-25 filter, Toyo Roshi Kaisha, Japan). The filter membrane was placed with cell side up on a YPD agar plate and incubated. For this experiment, we used YPD agar plates with different moisture levels, which were prepared as follows. When melted YPD agar was solidified with the lid closed, the agar surface was kept

**MATERIALS AND METHODS**

**ORGANISMS AND CULTURE CONDITIONS.** Heterothallic yeast strains used in this study were listed in Table 1. Genetic procedures were described elsewhere (Sherman et al., 1983; Suzuki and Yanagishima, 1986; Suzuki et al., 1989).

Rich media, YPD and YHG, were described by Sherman et al. (1983) and Suzuki and Yanagishima (1985), respectively. A synthetic minimal medium (SD) consisted of 2% glucose and 0.67% yeast nitrogen base w/o amino acids (Difco). Two % agar was added for solid media. For selective growth of transformant cells, appropriate amino acids were supplemented to SD agar. Temperature for cultivation and mating was 28°C. Liquid cultures were performed by shaking on a reciprocating shaker.
When YPD agar was solidified with the lid open for 15 to 20 min in a clean bench, humidity of the agar surface decreased. (2) Mating under pellet by spin condition: the cells were suspended in 3 ml YPD in a glass test tube (D = 14 mm) and centrifuged at 2000 rpm for 5 min, and then the test tube was incubated without agitation. (3) Mating under pellet by the natural gravity condition: the cells were suspended in 3 ml YPD in a glass test tube (D = 14 mm), and then the test tube was incubated without shaking.

After incubation under either one of the above conditions at 28°C for 6 hr, diploid cells were counted as follows. Each mixture was diluted with water and then portions were spread on SD agar. Auxotrophic haploid cells cannot grow on SD agar, while diploid cells can form colony because of complementation of auxotrophic mutations. After 2 days incubation, each colony that appeared on SD agar plates was counted as a diploid cell that had emerged at the mass mating condition. Total cell number was estimated by the colony formation on YPD agar.

To observe zygote cells microscopically, yeast cells were suspended in 1.85% formaldehyde/10 mM phosphate (pH 5.5) immediately after the mass mating reaction, then collected and re-suspended in the 10 mM phosphate buffer. Dumbbell-formed cells were estimated as diploid cells.
RESULTS

The allele agα1-1 in 1D1-16 strain (Suzuki and Yanagishima, 1985, 1986) is the mutation induced in KS13-1D by chemical mutagenesis. Agα1 gene was disrupted in this study by insertion of the URA3 gene in the Agα1 ORF as described in Materials and method. The resultant synthetic mutation agα1::URA3 was introduced into DBY746 and KK4 strains via recombination in vivo resulting in two mutant strains DBY746ag and KK4ag (Table 1). These agα1 mutant strains and their wild type parental strains were compared to reveal functions of sexual cell agglutination as the following.

On dry filter membrane, agα1 cells can mate as efficiently as wild type cells. Several conditions were employed here for conjugation (see Materials and methods). Cells were trapped on a filter membrane to appose MATα and MATα cells. Although agα1 cells mate well on the filter membrane, the efficiency was several-fold lower than that of the wild type cells (Table 2) as previously shown by Suzuki and Yanagishima (1985) and Lipke et al. (1989). This attenuated phenotype gave us vague answers to what are the physiological roles of sexual agglutination and of α-agglutination substance (Suzuki and Yanagishima, 1985; Lipke et al., 1989; see a review by Cross et al., 1988). During our many physiological experiments in this study, we found that the mutant cells sometimes showed a mating efficiency as high as that of the wild type. In contrast, the wild type (Agα1) cells always showed high efficiency of mating. This agα1-dependent fluctuation was found here to be due to moisture of the filter membrane. The membrane with yeast cells was placed on YPD agar for mating. When melted YPD agar had been solidified with the lid closed, the agar surface was kept moist. By this agar, mating efficiency of agα1 cells was 5–20 fold lower than that of the wild type. When YPD agar had been solidified with the lid open in a clean bench, humidity of the agar surface decreased. By this agar, mating efficiency of agα1 cells was raised up to the wild type level (Table 2).

To investigate the effect of moisture on mating of agα1 cells in detail, we observed cells microscopically because we preliminarily observed formation of longer zygotic cells in the mutant. As shown in Fig. 1C, length of zygotes formed on a membrane mounted on the moist YPD agar was longer than that of zygotes formed on a membrane on the less moist YPD agar, as we briefly mentioned before (Suzuki and Yanagishima, 1985). Contrarily, zygotes of the mutant formed on a membrane on the less moist YPD agar were found here to be as short as the wild type zygotes (Fig. 1D). The zygote length of the mutant formed on moist one had larger deviation. Length of wild type zygotes (Fig. 1A and 1B) was not affected by the difference of humidity of the agar used. The longer zygotes appear to be formed between gametic cells that are exposed to opposite mating type pheromone for unusually longer time probably because gametic cells failed to fuse with opposite mating type cells at a high frequency. These observations indicate that even a little water on the surface of the moist membrane disturbs intimate interaction between MATα cells and MATα cells, and the sexual cell agglutination is important to stabilize a-α cell pairs, while agα1 mutants can mate as efficiently as the wild type on a solid support of lower moisture. It is also suggested that mating reaction needs pressure or anchor to stabilize gametic cell pairs leading to zygote formation.

Sexual cell agglutination constructs multicellular structure to avoid overcrowding. Mating in pellet by the natural gravity was not good for both wild type and mutant cells. Even Agα1 cells, efficiency on this condition was lower by 100-fold than that on mating on filter membranes. In order to make a tighter cell contact, cells in the mating mixture were spun down by brief low speed centrifugation, and then kept still as described in Materials and Methods. In this pellet, the wild type cells mated at an efficiency one fifth of that on the filter conditions. Conversely, the mating efficiency of agα1 cells was significantly low under the pellet condition (Table 2). These results show that an intimate physical contact between MATα agα1 cells and MATα cells was established by pelleting whereas the contact was not favorable for mating reaction between MATα agα1 cells.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mating efficiency</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Filter membrane (dry)</td>
<td>1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Filter membrane (wet)</td>
<td>1.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Pellet by spin</td>
<td>0.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pellet by natural gravity</td>
<td>0.007</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1) For mating conditions in detail, see Materials and Methods section.
2) Mating efficiency was calculated by dividing a number of diploid cells by a number of diploid cells formed under the (dry) filter membrane condition.
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We found differences between the mutant pellet and the wild type pellet. A diameter of the pellet formed in mating between MATα agα1 cells and MATa cells was smaller than that in mating between wild type MATα cells and MATa cells. The pellet formed between MATα A Gα1 and MATa cells and the pellet formed between MATa agα1 and MATα cells are tentatively called large size pellet and small size pellet, respectively. The large type pellet was released from the bottom of the test tube much more easily than the small type pellet. The large type pellet was soft like cotton while the compact type pellet was tight and sticky like clay. Pellet types were compared using several laboratory strains and their agα1 derivatives. The difference in the pellet type characteristics between the wild type and the agα1 strains were also confirmed in these cells (Fig. 2). The size difference was observed in a broad range of cell number (Fig. 2A). Diameter of the
extended type pellet increased during the mating period 6 hr (Fig. 2B). After 8 hr incubation, the diameter rose 2 folds. In contrast, that of the compact type pellet remained almost unchanged (Fig. 2B). The characteristics co-segregated genetically with the $ag\alpha_{1}$ mutation in meiotic segregants from a heterozygous diploid strain (data not shown).

The above results suggest that the sexual agglutination forms a structure that can keep $MATa$ and $MAT\alpha$ cell mixture from overcrowding. The notions here were supported in the following experiments.

**Mating is enhanced by extending pellet.** The fact that pellet size is small and mating efficiency is low in the mating of $ag\alpha I$ cells let us to try to extend pellets in order to know whether the pellet form reflects the mating efficiency. Our prediction is that $ag\alpha I$ cells can mate better if cell pairs are stably immobilized but kept less overcrowded. As shown in Table 3, the mating efficiency was improved partially by substituting the test tube with one of larger size in diameter and by adding helper cells of $MAT\alpha$ mating type which had been killed by boiling but retain high sexual agglutinability. Addition of the helper cells increased the efficiency of the wild type cells 3-folds. By using larger size test tubes, the efficiency was increased 2-folds. Combination of the two alterations increased the efficiency additionally. In this respect also, large type pellet formation corresponded to the higher mating efficiency.

In the case of mating mixture of $ag\alpha I$ cells, addition of helper cells increased the efficiency more than 5-folds. Although in the case of $ag\alpha I$ cells the use of the larger size test tube extended the pellet size only a little and its effect on mating was uncertain, the combination of the two modifications to the mating condition increased mating efficiency more than 20-folds. Even in this case, the mating efficiency was still 1–2% of $AG\alpha_1$ cells, while the pellet size was increased 2-folds in diameter nearly to the level of that shown by $AG\alpha_1$ cells. It may be due to infrequent adhesion between $MATa$ and $MAT\alpha$ ag$\alpha_{1}$ cells because $MAT\alpha$ ag$\alpha_{1}$ cells without agglutinability would tend to be excluded out of the pellet-form aggregate of $MATa$ cells and boiled helper $MAT\alpha$ cells.

Since the promotive effect of the helper cells suggested a possibility that $\alpha$-agglutination substance stimulates $MATa$ cells, we checked the effect using partially purified $\alpha$-agglutination substance on conjugation of ag$\alpha_{1}$ cells. However, we could not detect increase nor decrease in mating efficiency by treating $MATa$ cells with the sub-

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**Table 3. Effect of extension of pellet on zygote formation.**

<table>
<thead>
<tr>
<th></th>
<th>KS13-1D ($AG\alpha_1$)</th>
<th>1D1-16 (ag$\alpha_{1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Help$^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>11.5</td>
<td>4.8 × 10$^7$/2.4 × 10$^7$</td>
</tr>
<tr>
<td>–</td>
<td>10.0</td>
<td>4.4 × 10$^7$/2.7 × 10$^7$</td>
</tr>
</tbody>
</table>

Mating was carried out under the pellet by spin condition using test tubes of $D=30$ mm (A) and those of $D=14$ mm (B).

1) Diameter of pellets measured at the end of mating reactions.
2) Number of diploid cells and number of total cells, measured at the end of mating reactions.
3) As helper cells, $4 \times 10^6$ cells of KS2-17A which had been killed by boiling were added to the mating mixture (+), or not added (–).

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Fig. 3. Cell pellets formed by various ascosporogenous yeasts. $4 \times 10^6$ cells harvested from overnight YPD cultures were suspended in 3 ml YPD in a testtube ($D=14$ mm). When two types of cells were mixed, $2 \times 10^6$ cells of each type were used. After centrifugation for 5 min at 2000 rpm, the bottom of the testtube was photographed to observe the pellet. The strains used were those listed in Table 1B.
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As shown above in Table 3, the addition of the helper cells increased also the mating efficiency of \( \text{AG} \alpha_1 \) cells, which have much \( \alpha \)-agglutination substance on their own cell surface. In addition, the fact that \( \text{MAT} \alpha \text{ag} \alpha_1::\text{URA3} \) cells mated under the dry membrane filter condition as efficiently as the wild type cells (Table 2) retreated the above possibility. Based on these observations, we concluded that the effect is physical one rather than chemical one.

Extended type pellet formation is mating specific phenomenon in ascosporogenous yeasts. We compared pellets of various combination of \( \text{MAT} \alpha \), \( \text{MAT} \alpha \), and \( \text{MATa/MATa} \) cells. After the cells were mixed and spun down, the pellet size was measured. As partly shown in Fig. 2B, all the pellets except one were of identical size (the compact type pellet). Only the pellet formed by a mixture of \( \text{MATa} \) cells and \( \text{MATa} \text{ag} \alpha_1 \) cells was the large extended type. The pellet by mixtures of \( \text{MATa} \) and \( \text{MATa} \text{ag} \alpha_1 \) cells were as small as pellets of non-mating mixtures (haploid cells of one mating type only (Fig. 2B), a mixture of \( \text{MATa/MATa} \) diploid cells and \( \text{MATa} \) cells and a mixture of \( \text{MATa/MATa} \) diploid cells and \( \text{MATa} \) cells (data not shown)). These results indicate that the extended type pellet formation is “mating specific” phenomenon. Because the extended type pellet was not formed by the mixture of \( \text{MATa} \) cells and \( \text{MATa} \text{ag} \alpha_1 \) cells, the sexual agglutination mechanism is responsible for the extended type pellet formation.

*Hansenula* and *Pichia* are the two major genera in ascosporogenous yeasts. Morphology of cell pellet was examined using 8 haploid strains in the two genera. As shown above in *S. cerevisiae*, the large type pellet formation was also observed in *Hansenula wingei*, *H. anomala*, *Pichia heedii* and *P. opuntiae* when two mating type haploid cells of own species were mixed (Fig. 3). It was strictly species specific (Table 4). The extended type pellets separated from the test tube bottom much more easily than the compact type pellets.

### DISCUSSION

Under the shaking condition in liquid media, \( \text{MATa} \text{ag} \alpha_1 \) mutant cells hardly adhere stably to the opposite mating type \( \text{MATa} \) cells because adhesion occurs only at random collision. Contrarily, it was assumed that cell contact is assured on filter membranes. However, the mutant cells showed clear differences in mating efficiency (Table 2) and in zygote length (Fig. 1) between moist and less moist filter membranes. The \( \text{ag} \alpha_1::\text{URA3} \) cells as well as \( \text{ag} \alpha_1-1 \) cells showed mating efficiency on the dry filter membrane as high as the wild type cells did and

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<table>
<thead>
<tr>
<th>Strain</th>
<th>SC</th>
<th>SC</th>
<th>HW</th>
<th>HW</th>
<th>PH</th>
<th>PH</th>
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<tbody>
<tr>
<td>T55(a)</td>
<td>S</td>
<td>L</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>T56(a)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>5(a)</td>
<td>S</td>
<td>L</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>21(a)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>503a(a)</td>
<td>S</td>
<td>L</td>
<td>S</td>
<td>S</td>
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<td>S</td>
</tr>
<tr>
<td>PH 503</td>
<td>S</td>
<td>L</td>
<td>S</td>
<td>S</td>
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<td>S</td>
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**Table 4. Species-specific extended pellet formation in ascosporogenous yeasts.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>HW</th>
<th>HW</th>
<th>HA</th>
<th>HA</th>
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<tbody>
<tr>
<td>5(a)</td>
<td>S</td>
<td>L</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>21(a)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>3(a)</td>
<td>S</td>
<td>L</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>8(a)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
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</table>

**Pellet type**: S, pellet diameter < 6 mm; L, > 8 mm (see Fig. 3).

The strains used were those listed in Table 1. Genera and species name were expressed in abbreviated forms as follows: SC, *S. cerevisiae*; HW, *Hansenula wingei*; HA, *H. anomala*; PH, *Pichia heedii*; PO, *P. opuntiae*. 
showed attenuated efficiency on the moist filter membrane. The attenuated data reflect the hyper fragile mating pair contact. It is preliminarily confirmed that $ag\alpha 1$ cells can mate efficiently if cell pairs are immobilized by pressing cells with a cover glass (Prof. Hisao Miyata's personal communication). These results propose two possibilities, namely, (1) start of cell wall fusion requires a time period between onset of cell adhesion and beginning of cell wall fusion, but excess moisture causes frequent changes of the mutual cell position in cell pairs most probably through the Brownian movement if gamete cells can not agglutinate, or (2) start of cell wall fusion requires force to push the counterpart cell surface in cell pairs by elongating mating tubes (Betz et al., 1981), but cells without agglutinability are unable to push each other on moist membrane filters because cells tend to slip off due to lack of anchors in excess moisture. We can not judge which idea is most probable. In any way, start of cell wall fusion event should need anchor or pressure to keep the contact by binding by agglutination substances.

The wild type large pellet formation found here was a mating specific phenomenon which is due to agglutinability of both $MAT\alpha$ and $MAT\alpha$ cells. Cappellaro et al. (1994) showed that $\alpha$-agglutination substance is a part of the outer fimbrial cell wall coat. The presence at the most outer surface is consistent with the phenomenon of the extended type pellet formation. The $ag\alpha 1$ cells were unable to form extended type pellet and showed significantly low mating efficiency under the pellet conditions. Even wild type cells increased efficiency using a larger test tube under the pellet condition. Too much compacted pellet and high cell density are not good for zygote formation. While the compact pellets of nonmating mixtures were tight, sticky and hard to be dispersed, the extended type pellet was easily separated from the bottom of the test tube as a single cotton-like flat mass. These results show that sexual agglutination including the extended type pellet formation offers $MAT\alpha$ and $MAT\alpha$ cells not only simple physical contact as a stick but also a uniquely organized structure favorable for zygote formation. Jackson and Hartwell (1990a, b) showed importance of pheromone concentration in courtship of $MAT\alpha$ and $MAT\alpha$ cells. Abnormal pheromone concentration may occur in the compact pellets due to too short distance between cells. In parallel, haploid cells must continue metabolism during mating processes as actively as in their vegetative state. The extended type pellet can afford the gametic cells with nutrients. It must be favorable also for growth of resultant zygote cells and subsequent vegetative diploid cells. The structure apposes and immobilizes cell pairs during the fusion event. Zygote cells retain agglutination substances, but vegetative diploid cells born from zygote cells do not have the substances (Yanagishima and Yoshida, 1981). This mechanism assures that diploid cells can easily make papillae on aggregates because the aggregates appears to have many vacant holes and the diploid cells are not immobilized by binding by agglutination.

Based on these results, we concluded that sexual cell agglutination contributes not only to specific adhesion and stabilization of cell pairs but also construction of a uniquely organized structure favorable for zygote formation, which ensures tight cell adhesion but avoid the risk of overcrowding, and beneficial for subsequent growth of diploid cells.

The mating specific large type pellet was also observed in Hansenula and Pichia yeasts, suggesting that the above notions revealed in $S. cerevisiae$ are also applicable in ascosporogenous yeasts. The precise structure constructed by $\alpha$ and $\alpha$ cells should be analyzed further.

This work is dedicated to the late professor Naohiko Yanagishima. The author is grateful to Professor Hisao Miyata (Nagoya Keizai University) for the permission to include the preliminary result as a personal communication.

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