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# A Biochemical Guide to Yeast Adhesins: Glycoproteins for Social and Antisocial Occasions

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## INTRODUCTION

Cell adhesion proteins are critical to fungal cell interactions in development, symbiosis, and pathogenesis. The adhesins are located on the surface of the cell wall, where they mediate the cell's interaction with the outside world. They participate in mating, colony morphology changes, biofilm formation, fruiting body development, and interactions with mammalian and plant hosts. These adhesins mediate two types of interaction, which can be thought of as "social" and "antisocial." The former type is represented by intraspecific interactions in mating and differentiation, such as those mediated by the *Saccharomyces cerevisiae* agglutinins and *Coprinus* galectins (78, 128), as well as formation of colonies and biofilms that adhere to and invade substrata (41, 42, 71, 89, 91, 110, 137, 142). On the other hand, those interactions we call "antisocial" are typical of pathogen binding to host organisms. Among these adhesins in ascomycetous yeasts are the *EPA* galectins of *Candida glabrata* and the hydrophobic cell surface proteins and peptide-binding Als proteins from *Candida albicans* (15, 25, 28, 33, 47). The Als proteins also mediate colony and biofilm formation, so they are "social" from the yeast's point of view and "antisocial" from the human standpoint. Such dual functions are common; proteins that evolved to facilitate cell interactions in fungal development may have been exploited to mediate interactions with host organisms in commensal and pathogenic situations (33, 34). Many of these activities have been recently discussed (124), so this review will emphasize the molecular architectures and binding properties that are used to mediate these functions.

### Structure of Adhesins

Yeast adhesins are of necessity mosaic proteins, because they need several domains with discrete functions. Their localization on the outer surfaces of thick cell walls determines the order of the domains and therefore determines their overall architecture. The domains are arranged in a standard order: N-terminal secretion signals, domains for ligand binding, optional central Thr-rich glycosylated domains that facilitate homotypic cell-cell interactions, N- and O-glycosylated stalks that elevate the binding domains above the wall surface, and C-terminal regions that mediate covalent cross-linking to the wall matrix through modified glycosylphosphatidylinositol (GPI) anchors. This review will concentrate on the proteins that conform to this model (Table 1), with particular emphasis on those

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TABLE 1. Properties of yeast adhesins

| Species | Adhesin (molecules per haploid cell) | Ligand (affinity[a]) | Known physiological function(s) | Reference(s) |
|---|---|---|---|---|
| *S. cerevisiae* | **a**-Agglutinin ($10^4$–$10^5$)[b] | α-Agglutinin ($10^{-9}$ M [r, p, c]) | Mating | 10, 41, 52, 106 |
| | α-Agglutinin ($10^4$–$10^5$)[c] | **a**-Agglutinin ($10^{-9}$ M [r, p, c]) | Mating | 10, 78, 134, 141 |
| | Flo1p | α-Mannosides | Flocculation | 5, 112, 116, 131 |
| | Flo5p | α-Mannosides | Flocculation | 4, 118 |
| | Flo9p | α-Mannosides | Flocculation | 41, 117 |
| | Flo10p | α-Mannosides | Flocculation | 41, 117 |
| | Flo11p ($10^6$)[d] | | Cell-cell adhesion in flocs, pseudohyphae, and biofilms; agar invasion; adhesion to plastic | 3, 41, 72, 81, 82, 101 |
| | Fig2p | Unknown | Mating | 41, 59, 138 |
| | Lg-Flo1p | | Flocculation | 67 |
| *C. albicans* | Als1p | | Aggregation, binding endothelia and epithelia | 27, 39, 47, 60 |
| | Als3p | | Biofilm formation | 90, 92, 142 |
| | Als5p | ($10^{-4}$ M to synthetic peptides [c]) | Aggregation, binding epithelia | 35, 49, 65, 99, 107 |
| | Other Als proteins | | | 38, 39, 47, 48, 50, 51, 139, 142, 143 |
| | Eap1p | Hydrophobic surfaces | Adhesion to plastic | 75, 76 |
| | Hwp1p | Substrate for tranglutaminase | Binds epithelia | 90, 110 |
| *C. glabrata* | Epa adhesins | β-Galactosides | Binds epithelia | 18, 21, 61 |

[a] Affinity estimates were derived by radioligand binding (r), plasmon resonance (p), and/or 50% inhibitory concentration for competitive ligand (c).
[b] Measured for *S. cerevisiae* strains W303-1A and X2180-1A.
[c] Measured for *S. cerevisiae* strains W303-1B and X2180-1B.
[d] Strain Σ1278b (Sungsu Lee and A. M. Dranginis, unpublished data).

for which the most information is available, namely, the *Saccharomyces cerevisiae* agglutinins and flocculins and the *Candida* Als proteins. Figure 1A illustrates hydrophobic cluster analysis (HCA) of the amino-terminal 440 amino acids of Flo1p (73). The amino acid sequence is helically wrapped, and residues are color coded by type. HCA analyses highlight repeated sequences as periodic patterns. Summary diagrams of several other adhesins are shown in Fig. 1B. The proteins are aligned at their C-terminal GPI anchor sequences to illustrate their attachment sites in the cell.

### A Word on Affinities

Throughout this review we describe binding affinities as being "low" (equilibrium dissociation constant [$K_D$] of $>10^{-6}$ M) (also called "weak binding") or "high" ($K_D$ of $\leq 10^{-6}$ M) (also called "tight binding"). The weak interactions are characterized by binding only when the adhesin and/or ligand is present at high concentrations, and any individual adhesin may bind to its ligand for only a few microseconds to seconds at a time, because dissociation rates tend to be high. In contrast, high-affinity interactions occur at low adhesin or ligand concentrations, and individual binding reactions may last for many minutes, corresponding to lower dissociation rates. Therefore, the strength and duration of adhesion between yeast cells or between the cell and its substrate vary by many orders of magnitude. In the lab, these differences are often seen as relative differences in resistance to vortexing or pipetting. In vivo there are great differences in resistance of adhesions to shear forces such as flow in the bloodstream or in biofilms in natural environments (105, 119).

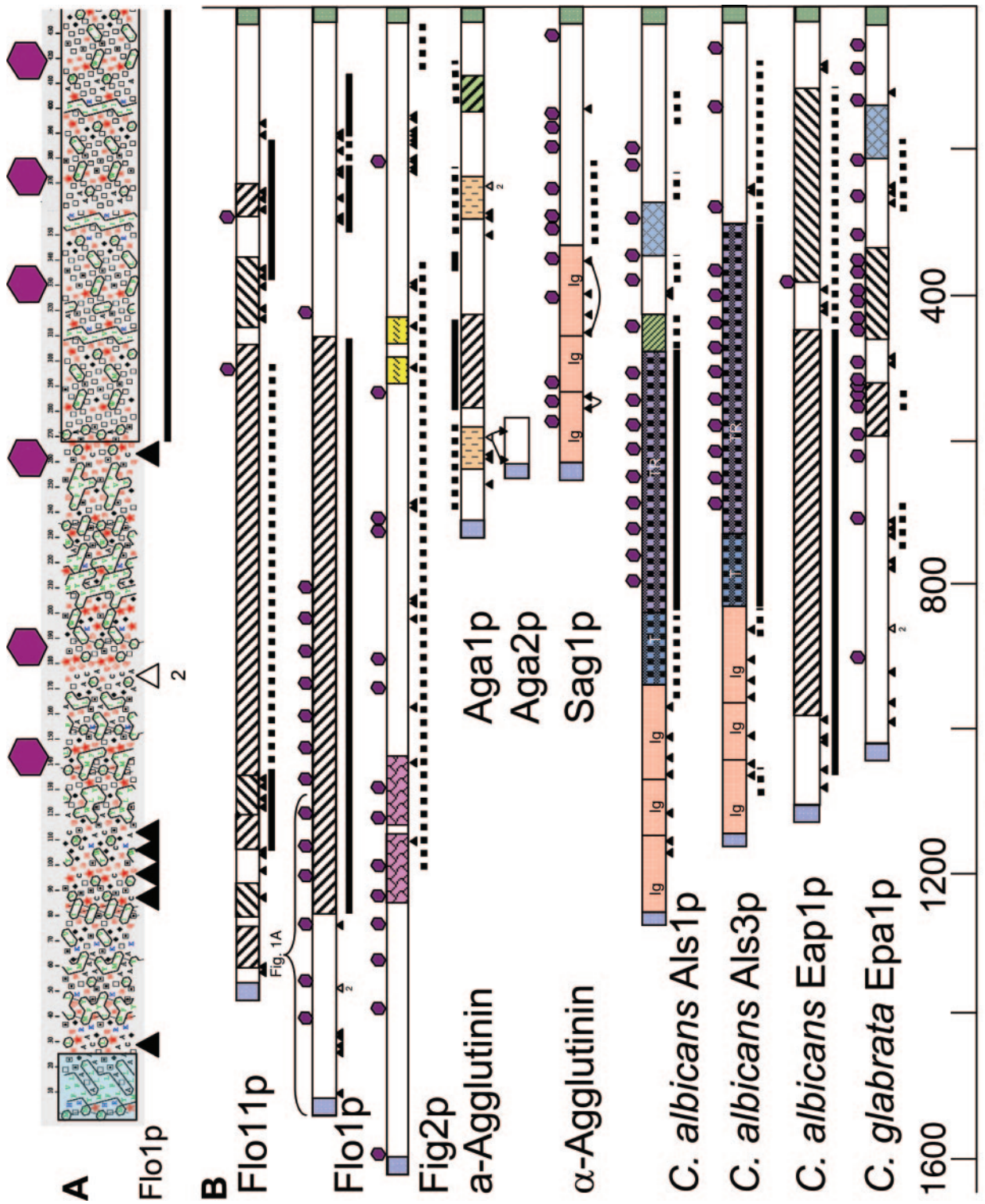
## ACTIVITIES OF ADHESINS

### The Adhesins of *Saccharomyces cerevisiae* in Mating

Among the "social" uses of adhesins, those of the benign bread yeast *S. cerevisiae* are probably the best studied. Each of the two mating types, **a** and α, expresses an agglutinin specialized for mating. Binding of these two agglutinins to each other on the surfaces of the mating cells facilitates fusion of the haploid cells (78).

***S. cerevisiae* a-agglutinin.** **a**-Agglutinin consists of two subunits, the products of unlinked genes *AGA1* and *AGA2*. **a**-Agglutinin has little similarity to other known adhesins. Aga1p is a 725-residue polypeptide with an N-terminal secretion signal sequence and a C-terminal GPI addition signal (Fig. 1). The remainder is Ser and Thr-rich and highly O glycosylated (103). Unexpectedly, Aga1p is expressed in both mating types, perhaps because of its role in agar invasion, as discussed below (41). The Aga2p subunit gives **a**-agglutinin its binding specificity and is expressed only on **a** cells. Although the mature form has only 69 amino acid residues, Aga2p contains all known binding determinants. This O-mannosylated glycopeptide is doubly disulfide linked to Aga1p (106). The Aga2p half-cystines are near the ends of the peptide, but they are linked to two Aga1p Cys residues separated by only two residues (106). Thus, the closeness of the disulfide bonds stabilizes an α/β structure in Aga2p, similar to small Cys-knot proteins (132).

Cell surface concentrations of **a**-agglutinin are low in most strains and are increased to about $5 \times 10^4$ per cell following treatment with the sex pheromone α-factor. The increased surface expression is a result of pheromone signaling through



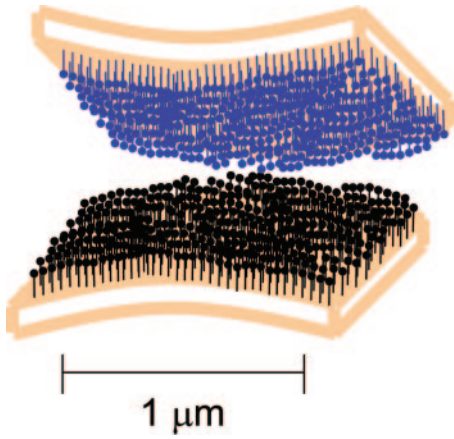



FIG. 2. Adhesin concentration in vivo. A scale drawing shows complementary 100,000-Da adhesins displayed on cell walls of two apposing yeast cells at the "moderate" surface concentration of about $2.5 \times 10^4$ molecules per cell, corresponding to a cell surface concentration of $\sim 4 \times 10^{-4}$ M. This surface concentration is approximately equivalent to that in a solution with 40 mg/ml of each of the adhesins, which is 100-fold greater than the usual concentrations in biochemical experiments with these proteins. The cell diameters are 4 μm, and a 1-μm by 1-μm section of each cell wall is shown as a curved tan sector with internal structure omitted. The lengths of proteins (~100 nm) are drawn to scale, but the thickness is exaggerated to improve visibility.

FIG. 1. Molecular features of representative yeast adhesins. These features are based on HCA plots of the adhesins. (A) HCA plot of the N-terminal 440 residues of Flo1p. HCA draws each open reading frame as a helical projection, which is vertically repeated. Individual amino acids are plotted and colored red for acidic, blue for basic, and green for hydrophobic, with hydrophobic patches bounded by black lines. Thr residues are drawn as hollow squares, Ser as dark-centered squares, Gly as diamonds, and Pro as red stars. Cys residues are marked with triangles below the HCA plot, and N-glycosylation sites are marked with maroon hexagons. Transparent boxes designate the N-terminal secretion signal (light blue) and the beginning of a tandem repeat region (open box). The line below the right-hand end shows that the Thr content of the repeat region is >25%. (B) Summary of HCAs of representative yeast adhesins, aligned at the C termini, where they are linked to cell wall polysaccharide through the GPI anchor remnant. N-terminal secretion signals are light blue, and GPI addition signals are green. Repeated sequences are boxed. Diagonal stripes in white boxes indicate tandem repeats that are not homologous to other repeats in the illustrated proteins. Where similar sequences recur, they are boxed and tinted in the same color. Potential N-glycosylation sites are shown as maroon hexagons above each open reading frame. Cys residues are shown as triangles below each open reading frame and are linked where the disulfide bonds have been mapped. The content of Thr is denoted by bars below each open reading frame, which are dotted where the Thr content exceeds 20% and solid where it exceeds 25%. (The frequency in the entire *S. cerevisiae* genome is 6%.)

a well-characterized mitogen-activated protein kinase pathway and increased transcription, translation, processing, secretion, and cross-linking to the wall (64). The Aga2p subunit binds with both low affinity and high affinity to α-agglutinin, which is expressed on the surfaces of cells of the opposite mating type, α.

C-terminal peptides of Aga2p are high-affinity ligands for α-agglutinin, and binding to the whole subunit is greatly reduced if additional amino acids are added at the C terminus (10, 106). This result implies that optimal binding includes interactions of the α-agglutinin binding pocket with the Aga2p terminal carboxyl group. Nevertheless, other regions of the **a**-agglutinin glycoprotein contribute to binding, either as secondary ligands or due to a role in maintaining proper conformation of the C-terminal peptide. The former interpretation seems more likely, because C-terminal fusions to Aga2p decrease affinity significantly but are still recognized by α cells (129). These low-affinity interactions will, in fact, occur at the cell surface, where local agglutinin concentrations can approach $10^{-3}$ M when the cell surface concentration is $10^6$ per cell (Fig. 2; Table 1) (106). Such high concentrations will effectively promote adhesin-ligand binding, even at very low affinities.

**α-Agglutinin.** α-Agglutinin consists of a globular head and a highly glycosylated extended stalk (10, 13, 78, 134). We modeled the globular region of α-agglutinin as three tandem immunoglobulin (Ig)-like folds, and the models have been tested by peptide mapping and circular dichroism (CD) analyses (13, 40, 77, 134, 140). Surface residues involved in ligand binding have been identified and mapped on the model (19). The Ig-like region of α-agglutinin is conformationally flexible: the native state has a high percentage of β-sheet (45%) and about 2% α-helix (13). The β-sheet content increases upon ligand

binding, and the α-helix content increases under environmental stress (140). Interestingly, the globular regions of the homologous Als proteins have been modeled more recently, and Ig-like templates were also used in that study (107).

**Interaction of the agglutinins.** Because both α-agglutinin and its ligand **a**-agglutinin have been extensively characterized, we can describe a model of the binding. Binding of α-agglutinin to high-affinity peptide epitopes on the **a**-agglutinin binding subunit Aga2p is slow and tight ($K_D$ of $\sim 10^{-9}$ M) (10, 106, 141). The agglutinins are displayed on the outer surface of the cell wall (10, 133) at $10^4$ to $5 \times 10^4$ copies per cell, corresponding to concentrations of 10 to 100 μM in the 100-nm space between the cells (Fig. 2) (106). At such concentrations, both high-affinity binding and low-affinity binding are rapid. Therefore, cell surface adhesion assays show interactions with micromolar or even larger dissociation constants. In contrast, in vitro binding assays such as surface plasmon resonance or $^{125}$I-protein binding assays can document only much tighter interactions, with affinities in the nanomolar to micromolar range. Indeed, the agglutinins bind with both nanomolar and micromolar affinities (106, 141). Because the C-terminal residues of Aga2p are the major binding determinant, a surface display model that fuses peptides to be screened to the C terminus of Aga2p inactivates the high-affinity binding (106, 129). Therefore, it is the low-affinity interactions that are used in screening. This result and mutational analyses demonstrate that the binding is complex, involving several regions of Aga2p with several regions of Agα1p (106).

In addition, conformational shifts in the agglutinins are essential for effective binding, even in this well-characterized, apparently simple reaction (141). Binding of the *S. cerevisiae* agglutinins is kinetically irreversible, due to very low dissociation rates (hours to days) (80, 141). This irreversibility would facilitate stable binding of partnered cells in mating and presumably results from interactions of individual molecules that include fairly large surface regions of each of the two agglutinins (19).

Such kinetic irreversibility is also characteristic of systems with multipoint attachments at lower affinity, such as flocculation or binding of Als proteins to many ligands on a mammalian cell surface. In these cases, multipoint attachments form as a result of the extremely high cell surface concentrations of the adhesins, many of which are bound to ligands that are also at high surface concentrations. For instance, flocculins bind to mannose residues, and Als proteins bind to common peptide sequence motifs, both with millimolar affinities. Mannose residues can be at molar or higher concentrations on the cell surface. If a cell is attached at two points on its surface, the dissociation rate of the cells decreases and the apparent affinity increases as the product of the individual values. Therefore, millimolar affinities become micromolar with two attachments, and nanomolar with three attachments, between the yeast and the multivalent ligand or substrate. If the dissociation rate for the binding was $10^{-1}$ $s^{-1}$, the corresponding whole-cell dissociation times would be about 10 s for a single-point attachment, 1.5 min for two attachments, and 15 min for three-point attachment. In this way, a high cell surface concentration of low-affinity adhesins and a lower concentration of high-affinity adhesins yield similar energetics and longevity of cellular adhesions.

**Other mating adhesins.** The **a**-agglutinin GPI-anchored subunit Aga1p has another adhesive role as well. While Aga1p functions as the anchorage subunit of **a**-agglutinin, it is expressed in both mating types. Aga1p or the similar protein Fig2p is essential for completion of mating and for mating-dependent invasion of agar (41). Little is known about whether these proteins act directly as adhesins, but Fig2p has a role in signal transduction to the wall stress pathway and therefore has a role in maintenance of cell wall integrity during mating (59, 138). It is pheromone induced and has an indirect role in attenuating sexual agglutination in mating type α cells (59, 138). Thus, Aga1p and Fig2p are partially redundant, and each has multiple adhesive roles. Such manifold activities and partial redundancy are common among the yeast adhesins in general.

## The *S. cerevisiae* Flocculins: Adhesins for Social Aggregation and Foraging

Many adhesins mediate interactions between yeast cells to form aggregates (flocs). These interactions have been called "flocculation" or "aggregation." We will use the former term to describe $Ca^{2+}$-dependent lectin-mediated interactions. Flocculation and aggregation are asexual; that is, they can occur among cells of any mating type and are not part of the mating response. These interactions are integral to biofilm formation (101).

*S. cerevisiae* harbors a family of flocculin genes with five members: *FLO1*, *FLO5*, *FLO9*, and *FLO10* (117) and *FLO11* (72, 82) Of these, only *FLO11* is expressed in most laboratory strains of *S. cerevisiae*, where it exhibits a profusion of phenotypes. *FLO11* expression is required for flocculation in *S. cerevisiae* var. *diastaticus* (82) and for invasion of substrates and formation of pseudohyphae in Σ1278b strains (72, 81). Flocculation is important to the brewing industry: Yeast cells flocculate spontaneously at the end of fermentation, with the result that the majority of cells are separated from the culture medium (122).

The Flo11p flocculin also enables yeast to adapt to a changing nutritional environment by switching to a pseudohyphal mode of growth. The filamentous chains of pseudohyphae (Fig. 3C), which may invade the substrate, develop in response to starvation for nitrogen in diploid cells. This mode of growth is considered an adaptation for this nonmotile species to forage for nutrients (36, 37). Pseudohyphal differentiation involves changes in gene transcription and cell cycle progression as well as changes in cell morphology (reviewed in reference 30). Flo11p may play a role in polarizing cell shape in addition to its role in adhesion (93). The Flo11p adhesin is required to hold the cells together in the branched chains. Thus, adhesin expression can govern colony morphology and invasion.

Flo11p also enables haploid yeast to invade agar in response to glucose starvation (17) or amino acid starvation (8). Adhesion to the substrate is the essential step in invasion (41). Presumably, invasion is then effected by the force of cell division from the adherent cells. In addition, *FLO11* is required for the formation of biofilm structures on agar (Fig. 3B) (101), for adhesion to plastic (101), and for formation of the specialized floating biofilms called flors that are responsible for the production of sherry wine (24, 54, 55, 137). The diverse phe-

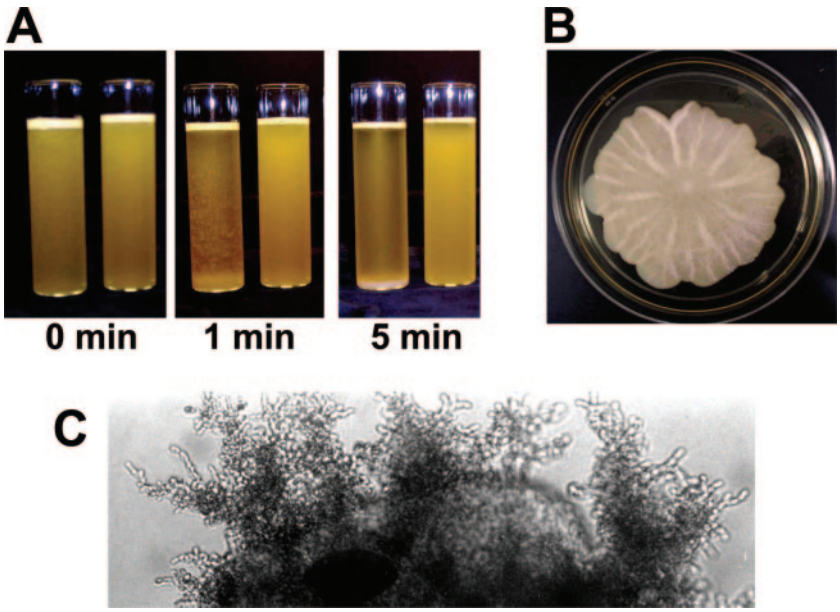



FIG. 3. Several phenotypes associated with the adhesin Flo11p. (A) Flocculation of *S. cerevisiae* var. *diastaticus* is Flo11 dependent. Yeast cultures of equal cell densities were vortexed vigorously and photographed at the indicated time intervals after mixing. Left tubes, wild-type haploid cells; right tubes, isogenic yeast with *FLO11* deletion. (B) *S. cerevisiae* strain Σ1278b form Flo11-dependent biofilms. A single colony of yeast is shown growing on semisolid medium in a standard 100-mm petri dish (101). The biofilm with its characteristic floral structure requires Flo11 for its formation. (C) Diploid Σ1278b strains form branched chains of cells called pseudohyphae in response to nitrogen starvation. Formation of these chains requires Flo11p (81).

notypes of *FLO11* could be explained as a consequence of altered adhesion in diverse circumstances. How different strains of yeast can exhibit different *FLO11*-dependent properties is a mystery still under investigation. For example, the *S. cerevisiae* var. *diastaticus* strain requires *FLO11* for flocculation (82) (Fig. 3A), while Σ1278b strains that express *FLO11* do not flocculate (41). Clearly, other factors must be involved in these phenomena. Potential causes might be sequence differences or differential glycosylation.

Flo1p and Flo5p, which are 96% similar (117), were identified as proteins that cause flocculation (4, 58, 116, 130, 131). They are large proteins that display structures typical of flocculins: a hydrophobic N terminus with signal sequence, a hydrophobic C terminus with a GPI anchor consensus sequence, and a central domain comprising tandem repeats of sequences that are extremely rich in Thr residues (Fig. 1). These are cell wall lectin-like proteins that participate directly in adhesive cell-cell interactions (4–6, 67, 118). Flo1p and Flo5p, along with Flo11p, have been identified as "Flo1-type flocculins" whose activity is inhibited by mannose but not by glucose (3, 108, 112). A variant flocculin, Lg-Flo1 has been identified as a member of the "NewFlo-type flocculins," a class whose activity is inhibited by both mannose and glucose. Domain swap experiments have established that the sugar-binding domains of Flo1p and Lg-Flo1 are the externally exposed N-terminal domains (67).

*FLO9* and *FLO10* were identified on the basis of their sequence similarity to *FLO1* and were found to also cause flocculation when expressed (41, 117). The *FLO* flocculins differ in their functional roles, but when overexpressed some can substitute for others. Expression from a galactose-inducible promoter of either Fig2p or Flo10p in strain Σ1278b causes agar invasion, like that caused by Flo11p. Overexpression of Flo1p does not cause agar invasion but does promote flocculation. Overexpression of Flo10p can promote both flocculation and Flo11p-like pseudohyphal development (41). Therefore, the roles of the flocculins are overlapping but not identical, and the flocculin family members have partial functional redundancy.

The flocculins are lectins, proteins that bind to cell surface carbohydrates. Each yeast lectin is specific for one or two monosaccharide haptens (e.g., for mannose or for mannose and glucose) but binds to haptenic oligosaccharides that contain these sugars in several different glycosidic linkages. Consequently, oligosaccharides with specific sugar in diverse linkages can act as hapten inhibitors (61, 124). Low specificity is usually accompanied by low affinity (millimolar or higher for monosaccharides and disaccharides) (56), because binding sites are broad and flexible, whereas high-affinity sites surround one specific kind of structure and maximize the interactions with all surfaces of the ligand. Broad specificity would allow binding to many structures on the surfaces of host cells and on other fungi.

### The *Candida albicans ALS* Family

The *C. albicans ALS* adhesins have both "social" (aggregative) and "antisocial" (pathogenic) functions. They have N-terminal Ig-like domains homologous to the *S. cerevisiae* sexual adhesin α-agglutinin (compare Als proteins and Sag1p in Fig. 1B) (47, 107). Als adhesins mediate adhesion to epithelia, yeast aggregation, and biofilm formation. The family is encoded at eight loci, and each locus is heterozygous (144). The various forms are expressed at different phases of growth and infection (47, 143). Because of the widespread expression of different

Als proteins in *C. albicans*, function can rarely be assigned to a single member of the family. Therefore, mechanistic studies have relied on heterologous expression in *S. cerevisiae*. In all known cases, Als-dependent behavior in *S. cerevisiae* mimics that in *C. albicans*, and *ALS1* and *ALS5* were cloned based on their ability to mediate *S. cerevisiae* adhesion to mammalian cells (27, 33, 107). Als1p is the most widely expressed member of the family and contributes to adhesion and colonization in an oropharyngeal candidiasis model (60). Als1p binds to endothelia and epithelia. Als3p is important for biofilm formation and induction of damage in an underlying oral epithelium (71, 90, 91, 100, 107, 142, 143). For the Als proteins, the N-terminal globular domain is necessary and sufficient for binding to cell surfaces (85, 99, 107). The binding specificity is extremely broad, and both Als1p and Als5p bind to peptides containing a common structural motif: τϕ+ (a turn-like residue, a bulky hydrophobic residue, and Lys or Arg). This motif is present in multiple copies in most proteins. Als5p and Als1p show overlapping specificities but show some differences in binding to individual instances of the motif (65). Peptides with other sequences also bind to Als proteins, making binding specificity even broader (32, 35). Flexibility of the peptide backbone is essential for binding, which is disrupted by H-bond perturbants (35). The apparent affinity to such peptides is around 0.5 mM. Thus, Als1p or Als5p causes expressing *C. albicans* to adhere to endothelia and epithelia, as well as biochemically defined substrates.

**Expression profile and allelic variation of *ALS* genes.** The *ALS* genes are differentially expressed in different growth phases and in different morphological forms (38, 48, 50, 51). *ALS1* expression is maximal just after inoculation into fresh growth medium (143). Similarly, *ALS3* expression is maximal when germ tubes are microscopically visible, and it may mediate the well-known autoaggregation of germ tubes (90, 143).

*ALS* gene activity accompanies *C. albicans* pathogenesis. Reverse transcription PCR tests detected *ALS* gene expression in human clinical specimens and in a vaginal candidiasis model (14). Although transcription from all *ALS* genes was observed, *ALS1*, *ALS3*, and *ALS9* were detected most frequently. Similar results for *ALS* transcriptional activity were found in a murine model of disseminated candidiasis (38). Experiments utilizing the gene encoding the yeast enhanced green fluorescent protein as a reporter gene under control of *ALS* promoters suggested that some Als proteins (mainly Als1p and Als3p) are abundant on the *C. albicans* cell surface, while others are produced at lower levels in the mouse disseminated candidiasis model (39).

Like the flocculins, Als proteins can aggregate cells that express them. Als-mediated aggregation does not require $Ca^{2+}$ and so is different from flocculation, which is $Ca^{2+}$ dependent. However, the Thr-rich domains of Als5p are clearly involved in cell aggregation in *C. albicans* (99). Therefore, the Als adhesins mediate both adhesion to mammalian tissues and aggregation of the *C. albicans* cells to form microscopic colonies. In parallel with a recent finding for *S. cerevisiae FLO1* (123), Als proteins with more copies of the Thr-rich repeats are more aggregative (47, 99).

### Other Adhesins *of C. albicans*

Several hydrophobic cell surface proteins increase partitioning of yeast cells into organic solvents in water/solvent interfaces. Of these, *C. albicans* Eap1p mediates binding to plastic surfaces in a manner resistant to shear forces (75). These properties are those required for stable adhesion to indwelling catheters and other devices, so the protein may be critical in prosthesis-induced candidemias and endocarditis. Eap1p has the same general architecture as the GPI-cross-linked lectins and peptide-binding proteins (Fig. 1). A hydrophobic-substrate-binding protein called Csh1p has been extensively functionally characterized in *S. cerevisiae* and, surprisingly, shows sequence similarity to known glycosyl transferases (45). Csh1p from *Candida dublinensis* functions similarly and has a similar distribution of hydrophobic residues in HCA analyses (not shown). Nevertheless, BLAST searches show its gene belonging to a different gene family; it is homologous to aryl oxidoreductases and $K^+$ channel regulatory domains (46). Neither of these Csh1p proteins possesses a secretion signal or a GPI addition signal. Rather, they are representative of a number of cytoplasmic metabolic enzymes that have historically been reported as cell wall constituents as well. Little is known of their localization or biological roles (45, 46, 66, 109). A number of homologs of each of these proteins appear in the *C. albicans* genome.

*C. albicans* Hwp1p, a glutamine-rich, GPI wall-anchored adhesin, is a substrate for epithelial cell transglutaminases (110, 111). Thus, it participates in a covalent cross-link between the yeast and the epithelium. The resulting association would be shear resistant, extremely close, and permanent in the absence of proteolysis. Such interactions could underlie the clinical observation that oropharyngeal colonies of *C. albicans* resist removal by scraping (89).

### *Candida glabrata*

The *C. glabrata EPA* lectins are members of the same gene family as the flocculins (BLAST E value of $10^{-11}$) and are also $Ca^{2+}$-dependent, but they bind β-galactosyl- and β-*N*-acetylgalactosaminyl-containing saccharides (15, 18). Therefore, they are members of the galectin class of sugar-binding proteins. The *EPA* gene family encodes adhesins which mediate binding to host epithelia during infections. While expression of *EPA1*, *EPA6*, and *EPA7* is repressed in the circulation of mice, these genes are specifically induced in the urinary tract, enabling *C. glabrata* to colonize the bladder (21). The mechanism of gene expression involves relief of telomeric silencing. The low nicotinic acid concentrations in urine lead to reduced activity of the $NAD^+$-dependent histone deacetylase Sir2p, which is necessary for maintenance of silencing. Levels of *EPA4* and *EPA5* were also observed to rise modestly in urine. In vitro, *EPA6* has been shown to be involved in biofilm formation (53).

## BROADENING THE SOCIAL LIFE: RECOMBINATION GENERATES DIVERSITY

Many of the adhesins include central-region direct repeats that are Thr rich (Fig. 1). Such repeats are highly vulnerable to

recombination, enabling the reshuffling of protein domains. The shuffling has led to diversity in adhesin structures and activities, as well as to "invention" of new proteins (123). In *S. cerevisiae*, four of the five *FLO* genes and their pseudogenes are adjacent to telomeres, a chromosomal location that is particularly susceptible to recombination. One such recombination event between the repeated sequences of *FLO11* and *SGA1* appears to have given rise to the *STA* gene family of secreted glucoamylases (72, 82, 126, 135). The *STA* family comprises *STA1*, *STA2*, and *STA3*, all of which are telomere-associated glucoamylase genes which are present only in the variant strain *S. cerevisiae* var. *diastaticus* (98). This variant yeast strain is defined by the presence of one of these genes. The genetic recombination that presumably gave rise to the *STA* genes resulted in the fusion of the amino-terminus-coding sequences (including the signal sequence and part of the Thr-rich repeats) of *FLO11* with the glucoamylase-coding sequences of *SGA1*. The resulting protein is a glucoamylase with a *FLO11* signal sequence and is secreted. *S. cerevisiae* var. *diastaticus* has been isolated on several occasions in different parts of the world from overfermented beer (1, 62, 113, 114). The ability to secrete glucoamylase enables the variant strain to utilize starch after other carbon sources have been exhausted. Thus, recombination of the Thr-rich repeats enables secretion, which confers a selective advantage over yeast without the *STA* gene.

Several repeated motifs in the *FLO* genes are conserved in the DNA sequence as well as in the amino acid-coding sequence, providing further evidence that homologous exchanges at these sequences may provide a selective advantage to the organism (125). Recombination between *FLO5* and a flocculin pseudogene may have given rise to the NewFlo-type flocculin Lg-Flo1p (67). The fact that most of the genes in the *S. cerevisiae* genome that contain repeated sequences encode cell wall proteins illustrates the functional significance of these domains (123).

The *ALS* adhesin genes of *C. albicans* also contain central domains of 108-bp tandemly repeated Thr-rich sequences, which exhibit considerable allelic variation. The number of these repeats varies considerably between alleles of a given *ALS* gene, resulting in a large repertoire of adhesins (47, 92, 144). Remarkably, 60 different alleles of *ALS7* were discovered in an analysis of 66 clinical isolates of *C. albicans*. The allelic differences were primarily due to rearrangements in repeated motifs (139). The genetic and allelic variabilities of *ALS* genes lead to differences in cellular behavior. Studies examining the two *ALS3* alleles showed a drastic difference in adhesion to vascular endothelial cells; the larger allele (containing a greater number of tandem repeats) conferred much more adherence than the smaller allele (92). Further analysis of *ALS* allelic pairing across five major *C. albicans* clades demonstrated a tendency of *C. albicans* to encode one smaller and one larger *ALS* allele (92).

Epigenetic regulation of flocculin gene expression provides additional variation in cell surface properties. When diploid *S. cerevisiae* cells are starved for nitrogen they develop as pseudohyphae, but this response is not homogenous. Some cells remain in the yeast form, which is the single-cell form. These yeast form cells have undergone an epigenetic switch that results in the metastable silencing of the *FLO11* gene (43). This switch is regulated by the histone deacetylase Hda1p and is heritable for several generations. The silent *FLO10* gene in these cells is also heterogeneously expressed, due to high-frequency mutations in *IRA1* and *IRA2*. Silencing of *FLO10* is accomplished by a different set of histone deacetylases, Hst1p and Hst2p (43). The consequent cell surface heterogeneity of yeast populations means that they are primed for rapid adaptation to changes in environmental conditions, because there are preexisting adhesive and nonadhesive subpopulations.

Thus, the repeat sequences generate genetic diversity in the flocculins through recombination, while epigenetic mechanisms provide the capacity for rapid adaptation to changing conditions. Given the short generation time of these unicellular organisms, a population of yeast is thus capable of rapidly changing its adhesive properties to permit colonization of new niches. Adaptive radiation of this sort may explain the periodic outbreaks of pathogenic strains of *S. cerevisiae* in patients when this usually harmless bread yeast is used as a probiotic therapy to control antibiotic-induced diarrhea (11).

## STRUCTURE OF GPI-ANCHORED ADHESINS

The GPI-anchored adhesins share a common architectural plan (Fig. 1), so we will discuss the various regions starting from the N terminus. In order, they are as follows: a secretion signal, a globular adhesion domain, an optional Thr-rich repeat domain, a glycosylated Ser/Thr-rich "stalk," the preanchor region, and the GPI addition signal. The lengths of GPI-anchored adhesins range from 650 residues (α-agglutinin) to about 1,650 residues for some of the Als proteins, Flo11p, and Fig2p.

### The N-Terminal Domains

N-terminal secretion signals comprise 20 to 30 amino acids and are cleaved by signal protease (10, 13).

The globular adhesion domains follow the signal sequences and contain the known ligand binding sites. In *S. cerevisiae* the α-agglutinin N-terminal region has the sedimentation coefficient of a globular domain and shows a CD spectrum typical of β-sheet-rich folds (115, 140). Residues important for binding to Aga2p are in this region and are localized in loops that connect β-strands in the Ig-like structural model (19). In *C. albicans* the Als protein N-terminal domains are homologous to α-agglutinin and so also have Ig-like structures. Als1p and Als5p also have β-sheet-rich CD spectra, and predictions have been made for ligand-binding regions (49, 107).

Other adhesion domains have not been physically studied, but secondary-structure studies are intriguing. CD spectroscopy shows that Aga2p has both β-sheet and α-helical regions (106). The Flo lectins are also predicted to be β-sheet rich by secondary-structure predictors. The prevalence of β-sheets in this small sample of adhesins may be fortuitous, but it might be a consequence of the acid stability of β-sheet structures in environments typical for growth of these acidogenic organisms (140).

### Central Thr-Rich Sequences

Many of the adhesins, including Flo lectins, Als adhesins, and Eap1p, have tandemly repeated central regions of several hundred residues that are rich in Thr and other β-branched amino acids (Fig. 1). Such amino acids have a strong preference for extended, β-sheet-like structures. However, the Pro residues also common in this region may prevent formation of compact domains, as would the many O mannosylations, which are present in the Thr-rich regions of Als5p and Flo11p. These regions have been proposed to function as spacers in Als proteins and in flocculins, holding the adhesion domain away from the wall (85). However, they also play additional roles in adhesion (99).

The Thr-rich repeats account for about 70% of the amino acids of Flo1. These repeats play a role in determining flocculation levels. In the flocculins and Als adhesins, proteins with fewer repeats display reduced flocculation levels (5). When the numbers of tandem repeats in *FLO1* were experimentally manipulated, a linear relationship was found between repeat number and flocculation as well as strength of adhesion to polystyrene (123). Similarly, the aggregation ability of the *C. albicans* Als proteins is roughly proportional to the number of Thr-rich repeats (99), and the Als5p repeats can mediate cellular aggregation (99). Therefore, it is likely that many of these repeats have adhesive activity as well as being recombinogenic.

### Ser/Thr-Rich Stalks

The Ser/Thr-rich regions are at least 300 residues long, with 35 to 55% Ser and Thr residues. In the few cases where they have been analyzed for carbohydrate content (the Aga1p analog from *Hansenula wingei*, the first 25 residues of the Ser/Thr region in α-agglutinin, and a similar region near the C terminus of the GPI membrane protein Gas1p), all Ser and Thr residues have been found to be O glycosylated (13, 31, 136). Thus, they would be predicted to form an extended conformation (57). Such an extended structure is consistent with the predicted length of the stalk visible in an electron micrograph of wall-bound α-agglutinin (10, 78). This length would allow the ligand-binding regions of Epa1p, Als1p, and Aga2p to be displayed far enough from the wall surface to be active (78). In these cases, deletion of some amino acids from this region results in inactive adhesins (26, 52, 85, 106). In an elegant study, Frieman and Cormack showed that when this region was deleted from Eap1p, the adhesion domain was present in the wall but was not sufficiently exposed to bind to its ligand because the stalk was too short (26).

Two features of the Ser/Thr-rich C-terminal regions are common but have unknown consequences, and their roles have not been systematically tested. First, all Ser/Thr-rich regions also have substantial clusters of hydrophobic residues (Fig. 1). Second, the C-terminal regions of the Ser/Thr regions are often rich in Cys residues as well. These Cys residues form a disulfide network near the surface of the wall, and this network limits wall permeability (Fig. 1) (20, 63, 64). Such a region in Aga1p helps to cross-link the adhesin to the wall, presumably through bonds to Cys residues in other GPI-anchored proteins (52, 64, 106).

### Pre-GPI and GPI Signal Sequences

GPI-linked proteins in fungi are in two classes. Some, typified by Gas1p, are primarily membrane proteins and tend to remain with their GPI fatty acids embedded within the membrane bilayer. On the other hand, the adhesins and other wall proteins, such as Cwp proteins in *S. cerevisiae*, lose their association with the membrane, and become covalently cross-linked to the wall glucans. In α-agglutinin and other GPI wall proteins, a truncated GPI glycan becomes transglycosylated to β-1,6-glucan (63, 69, 79).

There is significant research addressing the question of whether there is also a signal that specifies that a GPI moiety will be directed to the wall or remain membrane attached. Investigation of this question is complicated by the observation that GPI proteins are always found to some extent in both locations (16, 87, 88). Indeed, recent information shows that GPI-linked wall proteins are often cross-linked into the wall by other mechanisms as well, including glutamine-dependent transesterification (22, 64) and disulfide bond formation (52). The result of these complications is that the proportion of proteins localized within the wall has usually been underestimated, a result of incomplete release of the wall-bound proteins as well as because of poor electrophoresis and blotting of yeast proteoglycans (88). Nevertheless, the sequence immediately before the GPI addition signal influences the ratio of membrane-associated to wall-associated protein (9, 44). Specifically, hydrophobic residues at positions 4 and 5 before the GPI transpeptidylation site direct a greater proportion of the protein to the wall (9), and basic residues at one and two residues before this site direct more protein to the membrane (64). HCA plots show that all "wall-directed" proteins have substantial hydrophobic regions four to eight residues N terminal to the GPI addition site, whereas the "membrane-directed" proteins show an exclusion of hydrophobic residues within about 20 residues of the site (not shown).

## CONCLUSIONS

Yeasts are nonmotile eukaryotes that rely on their adhesins for selective interaction with the environment and with other cells. We have reviewed the structure and binding properties of yeast adhesins. We have not attempted to address all areas of the adhesin literature. Notably absent, for example, is a discussion of the large and growing body of literature on the many regulatory pathways that govern expression of the adhesins (for reviews, see references 71, 74, 76, 91, and 124). *FLO11* gene expression, for example, is regulated by many signaling pathways (94), including the cyclic AMP/protein kinase A pathway (2, 70, 95–97, 102, 127), a mitogen-activated protein kinase pathway (29, 86, 104, 121), nutritional sensing pathways (8, 17, 37, 68, 84, 120), a quorum-sensing pathway (12), and cyclins (83). Perhaps because of this, the *FLO11* promoter is one of the largest known in yeast, as befits a gene that must respond to diverse circumstances.

The common structural motifs of the adhesins described here suit adhesin function extremely well. The glycoproteins are secreted to the outer face of the plasma membrane, then they are covalently anchored in the wall with their binding domains elevated beyond the wall surface (10, 26, 79). Their

N-terminal globular domains bind peptide or sugar ligands. Central domains often include tandemly repeated sequences that are highly glycosylated. These Thr-rich domains potentiate cell-to-cell binding, but the molecular mechanism of such an association is not yet clear. These repeats also mediate recombination between repeats and between genes. The high levels of recombination and epigenetic regulation are sources of variation which enable the population to continually exploit new niches and resources.

While the structural motifs described in this review are widespread among the adhesins, they are not universal. Among the adhesins with alternative structures is Bad1p, for example, a molecule secreted by *Blastomyces dermatiditis* which utilizes an epidermal growth factor-like domain to bind cells and downregulate the immune response (7).

The adhesins are subjects of medical interest, since adhesion to tissue is an obligatory first step in pathogenesis by many yeasts. These yeasts also use adhesins to colonize catheters and other prosthetic medical devices and thus produce biofilms. Within biofilms, organisms are quite resistant to antimicrobial therapy, frequently necessitating removal of the plastic medical device. Fungal pathogenesis is emerging as a significant cause of infection due to the increase in frequency of chronic predisposing factors such as human immunodeficiency virus infection, cancer chemotherapy, corticosteroid therapy, and use of broad-spectrum antibiotics (23). The cell wall exposure of the adhesins make them attractive candidates for targeted drug therapy to interfere with adhesin function or with their anchorage in the cell wall. This interference would disrupt social networks among the fungi and improve them in the host organisms.

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