Review

Lars-Oliver Essen*, Marian Samuel Vogt and Hans-Ulrich Mösch*

Diversity of GPI-anchored fungal adhesins

<https://doi.org/10.1515/hsz-2020-0199>

Received May 29, 2020; accepted September 21, 2020;

published online October 9, 2020

Abstract: Selective adhesion of fungal cells to one another and to foreign surfaces is fundamental for the development of multicellular growth forms and the successful colonization of substrates and host organisms. Accordingly, fungi possess diverse cell wall-associated adhesins, mostly large glycoproteins, which present N-terminal adhesion domains at the cell surface for ligand recognition and binding. In order to function as robust adhesins, these glycoproteins must be covalently linked to the cell wall via C-terminal glycosylphosphatidylinositol (GPI) anchors by transglycosylation. In this review, we summarize the current knowledge on the structural and functional diversity of so far characterized protein families of adhesion domains and set it into a broad context by an in-depth bioinformatics analysis using sequence similarity networks. In addition, we discuss possible mechanisms for the membrane-to-cell wall transfer of fungal adhesins by membrane-anchored Dfg5 transglycosidases.

Keywords: cell adhesion; domain structure; evolution; fungal cell wall; glycoproteins; protein families.

Introduction

In fungi, selective cell adhesion is fundamental for numerous biological processes including multicellular

development, social interactions, colonization and pathogenesis. As true for other microbes, adhesive behaviour of fungal cells is determined by the surface properties of the outer cell wall. A large body of evidence has shown, that the cell wall of fungi is a highly specialized and dynamic compartment that not only confers contact with the environment, but also plays a crucial role in maintenance of osmotic integrity, determination of cell morphology and protection against physical stress and mechanical damage (Garcia-Rubio et al. 2019; Gow et al. 2017; Orlean 2012). In order to fulfil these diverse functions, fungal cell walls have highly complex structures that are composed of ordered layers consisting of glucans, chitin, chitosan and glycosylated proteins. Extensive genome-wide studies in *Saccharomyces cerevisiae* and other yeasts have shown that approximately 20 % of the fungal genome is involved in cell wall structure, function, biosynthesis and dynamics, underscoring the fundamental importance of this highly complex organelle for fungal growth and development (de Groot et al. 2003; Lesage and Bussey 2006).

Cell wall-associated proteins (CWPs) are usually polysaccharide-linked glycoproteins, which fulfil a plethora of functions including adhesion, absorption of molecules, signal transduction and protection as well as synthesis and reorganization of wall components and anchoring of cell wall proteins (Bowman and Free 2006). With respect to cell adhesion, a growing number of fungal CWPs have been identified that have been demonstrated or postulated to act as cell surface adhesins and confer binding to diverse ligands (Lipke 2018). To date, the majority of functionally characterized fungal adhesins are glycoproteins with a common modular architecture (Figure 1) that is characterized by (i) a cell surface exposed N-terminal adhesion domain (A domain) for ligand recognition and binding, (ii) a large middle segment carrying the A domain and consisting of variable numbers of glycosylated, serine- and threonine-rich repeats (B domain), and (iii) a C-terminal domain harbouring a glycosylphosphatidylinositol (GPI) anchor mediating attachment to the glucan layer of cell walls by transglycosylation (de Groot et al. 2013). Therefore, this group of fungal glycoproteins has been named GPI-CWP adhesins (Dranginis et al. 2007), most of which have been found in ascomycetes originating from the genera of *Saccharomyces*

***Corresponding authors:** Lars-Oliver Essen, Department of Biochemistry, Faculty of Chemistry, Philipps-Universität Marburg, Hans-Meerwein-Straße 4, D-35043 Marburg, Germany; and Center for Synthetic Microbiology, Philipps-Universität Marburg, Karl-von-Frisch-Str. 6, D-35043 Marburg, Germany, E-mail: essen@chemie.uni-marburg.de. and

Hans-Ulrich Mösch Department of Genetics, Philipps-Universität Marburg, Karl-von-Frisch-Str. 8, D-35043 Marburg, Germany; and Center for Synthetic Microbiology, Philipps-Universität Marburg, Karl-von-Frisch-Str. 6, D-35043 Marburg, Germany, E-mail: moesch@staff.uni-marburg.de, <https://orcid.org/0000-0002-4660-6070>

Marian Samuel Vogt, Department of Biochemistry, Faculty of Chemistry, Philipps-Universität Marburg, Hans-Meerwein-Straße 4, D-35043 Marburg, Germany. <https://orcid.org/0000-0002-9867-3603>

and *Candida* in the subphylum of Saccharomycotina (Lipke 2018). Comparative genomic analysis has revealed that the number of genes for GPI-CWP adhesins present in different fungal species can be highly variable ranging from less than 10, as exemplified by the budding yeast *Saccharomyces cerevisiae* (Brückner and Mösch 2012) or non-pathogenic *Candida* species (Gabaldon et al. 2013), to much higher numbers as found for the most frequently isolated human fungal pathogens *Candida albicans* (de Groot et al. 2013) or *Candida glabrata* that contains more than 80 members (Timmermans et al. 2018; Xu et al. 2020).

Understanding the diversity and structure-function relationships of GPI-CWP adhesins significantly contributes to answering basic biological questions, including on

how fungal glycoproteins have evolved to mediate selective adhesion during multicellular development and to confer kin discrimination (Fisher and Regenbergh 2019). In addition, GPI-CWP adhesins are crucial virulence factors that support and enable fungal infections, thus underscoring the importance of these proteins to human health (Brown et al. 2012; de Groot et al. 2013). It has for instance been discussed that an increase in the number of GPI-CWP adhesin genes might reflect evolutionary adaptation to the human host (Gabaldon et al. 2013).

In recent years, an increasing body of research on GPI-CWPs has been performed to (i) enable gene discovery by comparative genomics, (ii) study gene regulation by genome-wide expression analysis, (iii) elucidate physiological roles by molecular genetic approaches using original organisms and heterologous systems, (iv) characterize biochemical functions by glycan array or peptide screening and subsequent quantitative *in vitro* binding analysis, (v) determine three-dimensional structures by X-ray crystallography and NMR, and (vi) reveal biophysical properties by single cell atomic force microscopy. Nonetheless, only a limited number of GPI-CWPs has been studied in detail at all levels, which so far include members of the families of PA14-type, Flo11-type and Als-type adhesins (Table 1). In addition, a growing number of GPI-CWPs have been annotated as adhesins, that have not been functionally analysed yet. In this review, we will focus on functionally well-studied GPI-CWP adhesion domains by highlighting recent studies on yeast models and by discussing the potential functions of members that lack experimental characterization, many of them belonging to filamentous and dimorphic fungi. By using sequence-similarity network analyses covering the whole fungal kingdom we are now also capable to provide an overview of the occurrence and variation of the different protein families of fungal adhesion domains. As such, our review complements recent reviews on fungal adhesins that focus on genomics (Lipke 2018), protein structure and interactions (Willaert 2018) or cellular aggregation (Lipke et al. 2018).

It is important to point out, that functionality of fungal adhesins not only depends on the presentation of suitable adhesion domains at the cell surface, but equally on stable anchoring (Frieman and Cormack 2003; Frieman et al. 2002; Huang et al. 2003; Lu et al. 1995). In the case of GPI-CWP adhesins, anchoring is mediated by the GPI moiety that is added to the C-terminal domain during secretion (de Groot et al. 2003). Upon arrival at the plasma membrane, anchoring is achieved by a lipid-to-wall transfer step that involves transglycosylation to glucans and is catalysed by specific glycoside hydrolases of the

Figure 1: General architecture and cell wall transfer of fungal GPI-anchored adhesins.

The N-terminal domain for adhesion to glycans or peptides (A) is shown in magenta, the highly glycosylated and repetitive middle domain (B) is presented in beige, and the C-terminal domain harbouring the GPI-anchor (C) is shown in brown. The cell wall (CW) is shown in pink with the beta-glucan network presented in purple and the chitin layer in light blue. The plasma membrane (PM) and cytoplasm (CP) are indicated. Further details are described in the text.

Table 1: Structural and functional properties of fungal adhesion domains.

Class	Adhesion domain	Organism	Physiological roles	Physiological targets	Preferred ligands	Core structure/ binding pocket	Number of Cys/SS bridges	Structural signatures
Flo-type	ScFlo1A ¹	<i>S. cerevisiae</i>	Fungal cell aggregation	Mannoproteins	Man α -linked glycans	PA14 ² /GBP ³	8/3	DD-N ⁴ , Flo-type subdomain
	ScFlo5A ⁵	<i>S. cerevisiae</i>	Fungal cell aggregation	Mannoproteins	Man α -linked glycans	PA14/GBP	8/3	DD-N, Flo-type subdomain
	LgFlo1A ⁶	<i>S. pastorianus</i>	Fungal cell aggregation	Mannoproteins	Man α - and Glc α -linked glycans	PA14/GBP	8/3	DD-N
Epa-type	CgEpa1A ⁷	<i>C. glabrata</i>	Host cell adhesion	Epithelia, fibronectin, mucin	Gal β -linked glycans	PA14/GBP	8/4	DD-N, W-R ⁸ , CBL2 ⁹
	CgEpa6A ¹⁰	<i>C. glabrata</i>	Host cell adhesion	Epithelia	Gal α - and Gal β -linked glycans	PA14/GBP	8/4	DD-N, W-R, CBL2
	CgEpa9A ¹¹	<i>C. glabrata</i>	Host cell adhesion	Epithelia	Gal α - and Gal β -linked glycans	PA14/GBP	8/4	DD-N, W-R, CBL2
Cea1-like	KpFlo1A ¹²	<i>K. pastoris</i>	Unknown	Chitin	GlcNAc β -linked glycans	PA14/GBP	8/3	DD-N, CBL2
Flo11-type	KpFlo2A ¹²	<i>K. pastoris</i>	Unknown	Unknown	Unknown	PA14/GBP	8/3	DD-N, CBL2
	ScFlo11A ¹³	<i>S. cerevisiae</i>	Biofilm formation, kin discrimination	Flo11-type adhesins	ScFlo11A	FN3 ¹⁴	6/3	Aromatic bands
	KpFlo11A ¹⁵	<i>K. pastoris</i>	Biofilm formation, kin discrimination	Flo11-type adhesins	KpFlo11A	FN3	6/3	Aromatic bands
Als-Type	CaAls3A ¹⁶	<i>C. albicans</i>	Host cell adhesion	Endothelia, epithelia, fibronectin, laminin, collagen, <i>Staphylococci</i>	Hexapeptides, C-terminal carboxylate	IgG ¹⁷ /PBC ¹⁸	8/4	K ¹⁹
	CaAls9A ²⁰	<i>C. albicans</i>	Bacterial cell adhesion Host cell adhesion	Epithelia, laminin, fibrinogen	Hexapeptides, C-terminal carboxylate	IgG/PBC	8/4	K

¹Goossens et al. (2015). ²Protective antigen 14 domain from anthrax toxin of *Bacillus anthracis* (Petosa et al. 1997; Rigden et al. 2004). ³Glycan-binding pocket (GBP). ⁴Highly conserved DcisD motif in close spatial vicinity to a conserved asparagine residue conferring Ca²⁺ and glycan binding within the GBP (Veelders et al. 2010). ⁵Veelders et al. (2010). ⁶Sim et al. (2013). ⁷Maestre-Reyna et al. (2012). ⁸Highly conserved tryptophan residue in close spatial vicinity to a conserved arginine residue conferring high-affinity ligand binding within the GBP (Maestre-Reyna et al. 2012). ⁹Calcium-binding loop 2 (CBL2) carrying highly variable residues conferring ligand binding specificity within the GBP (Maestre-Reyna et al. 2012). ¹⁰Diderrich et al. (2015). ¹¹Diderrich et al. (2015), Hoffmann et al. (2020). ¹²Kock et al. (2018). ¹³Kraushaar et al. (2015). ¹⁴Fibronectin-type 3 domain. ¹⁵Brückner et al. (2020). ¹⁶Lin et al. (2014). ¹⁷Immunoglobulin G-type domain. ¹⁸Peptide-binding cavity (PBC). ¹⁹Highly conserved lysine residue at the end of the PBC conferring binding to the C-terminal carboxylate of peptides (Salgado et al. 2011). ²⁰Salgado et al. (2011).

class 76 (GH76) family (Kitagaki et al. 2002). So far, only a few fungal genes belonging to the Dfg5/Dcw1 family have been identified, which are involved in adhesion and cell wall anchoring and that have been suggested to act as

enzymes catalysing the membrane-to-wall transfer of GPI-CWP adhesins (Ao et al. 2015; Maddi et al. 2012; Mösch and Fink 1997; Muszkieta et al. 2019; Spreghini et al. 2003). Accordingly, we discuss the mechanisms of GPI-adhesin

maturation and incorporation into the cell wall given our current understanding of fungal GPI biosynthesis and remodelling (Kinoshita and Fujita 2016; Komath et al. 2018).

PA14-type adhesins

Functionally and structurally, the best-characterized GPI-CWPs are fungal adhesins comprising an exposed PA14-type domain (PFAM entry PF07691). This group is exclusively found in ascomycetes. Functional *in vivo* and *in vitro* analysis has been performed on more than 20 different PA14-type GPI-CWPs revealing that their N-terminal A domains are lectins that confer cell-cell and cell-substrate adhesion by specific binding of diverse glycan ligands in a C-type lectin-like manner. In addition, nine different 3D structures of PA14-type domains have been reported so far from the *S. cerevisiae* flocculins Flo5 and Flo1 (Goossens et al. 2015; Veelders et al. 2010), the *C. glabrata* epithelial adhesins Epa1, Epa6 and Epa9 (Diderrich et al. 2015; Hoffmann et al. 2020; Ielasi et al. 2012; Maestre-Reyna et al. 2012), two LgFlo1 flocculins from *Saccharomyces pastorianus* strains (Goossens et al. 2015; Sim et al. 2013) and two *Komagataella pastoris* PA-14 domains, including the chitin-end binding adhesin Cea1 (Kock et al. 2018). The common features of these adhesion domains are (i) a β -sandwich core structure related to the bacterial PA14 domain from anthrax toxin (Petosa et al. 1997; Rigden et al. 2004), (ii) a calcium-dependent carbohydrate-binding pocket carrying a unique DcisD motif and two calcium binding loops (CBL1, CBL2), and (iii) the ability to recognize the non-reducing disaccharidic ends of glycans present on neighbouring cells or host surfaces.

In the current INTERPRO database (April 2020), PA14-type domains can be found in 791 fungal cell wall proteins. A previous structure-based phylogenetic analysis using a limited number of PA14-type GPI-CWP adhesins has uncovered six different clusters (Kock et al. 2018). We here provide a more comprehensive analysis of all fungal PA14-type adhesion domains by employing the Enzyme Similarity Tool (EFI-EST) (Gerlt et al. 2015), which provides a protein sequence similarity network (SSN) that visualizes broad relationships (Figure 2). The network analysis reveals that fungal PA14-type adhesion domains can be divided into two major groups, depending on the presence of two disulphide bridges, which crosslink the N- and C-terminal ends to the core domain. As can be seen in the middle panel of Figure 2B, Group I is mostly restricted to Saccharomycetes and includes the flocculin-related (Flo-

type) cluster, the epithelial adhesin-related (Epa-type) cluster, a cluster comprising several Saccharomycetaceae species like *Kluyveromyces marxianus* or *Kluyveromyces lactis* (Kluy-type) and several adhesion proteins from Phaffomycetaceae (Kock et al. 2018). Group II consists of a large cluster of fungal PA14-type domains (CC-less), which lack a conserved motif of two consecutive cysteine residues present in the first group. Members of Group II are generally not located at the N-terminus like the aforementioned groups and often lack the DcisD motif required for calcium binding. The special features of these groups and clusters are discussed in the following paragraphs with a focus on the known physiological roles and structure-function relationships of well-characterized representatives.

Flo-type cluster

The Flo-type cluster currently contains 124 members from 11 species including a multitude of *S. cerevisiae* strains. The best characterized members of this cluster include the PA14-type flocculins Flo1, Flo5, Flo9 and Flo10 from *S. cerevisiae* and Lg-Flo1 from *S. pastorianus*, which all confer calcium-dependent aggregation of thousands of yeast cells into flocs by specific binding of mannoproteins present at the cell surface of (Brückner and Mösch 2012). Floc formation has been studied for decades and is of considerable importance in the brewing and wine-producing industry (Bauer et al. 2010; Van Mulders et al. 2010). In natural environments, the role of these multicellular aggregates is likely to confer protection against harmful agents and conditions (Smukalla et al. 2008). In addition, flocculin genes fulfil the definition of green-beard genes that confer benefits to cells or organisms that carry this allele (Dawkins 1976; Hamilton 1964). In the case of flocculins, self-nonself discrimination is ensured by the simple fact, that two-way bonding between carrier cells both presenting a FloA domain is much more efficient than one-way bonding between carrier cells and cells not expressing the adhesin (Veelders et al. 2010). This difference in binding strength causes cell sorting during flocculation and results in enrichment of non-expressers, which fail to invest into the cost of adhesin production, at the non-protective periphery of flocs and a corresponding selective disadvantage to such cheaters (Smukalla et al. 2008).

So far, crystal structures of the A domains of Flo1, Flo5 and LgFlo1 in complex with different mannosides have revealed the structural basis for ligand binding and discrimination for Flo-type adhesins (Goossens et al. 2015;

Figure 2: Sequence-similarity network of fungal PA14 adhesins.

(A) Fungal PA14 adhesins are part of the large PA14 domain superfamily (INTERPRO entry IPR0037524; shown as inset, E-value cut-off 10^{-30}); whose 13558 members are mostly of bacterial origin and part of surface proteins or glycan-processing enzymes. 360 of the 795 fungal PA14 adhesin-like gene products (SSN shown with E-value cut-off 10^{-35}) belong to the fungal class of Saccharomycetes; the remainder to filamentous fungi and other, which mostly lack the *DcisD* motif. Currently, all known structures belong to Saccharomycetes and harbour a *DcisD* motif as required for putative C-lectin like functions. Abbreviations: *Cg*, *Candida glabrata*; *Sc*, *Saccharomyces cerevisiae*; *Sp*, *Saccharomyces pastoris*; *Kp*, *Komagataella phaffii*. SSN in Figures 2–4 were generated by EFI-EST using UniProt release 2019_10 (Zallot et al. 2019) and analysed by Cytoscape v3.8.0 (Shannon et al. 2003). (B) Mapping of key structural features to fungal PA14 domains like the presence of an intact *DcisD* motif (left), the CC motif responsible for disulphide-bridged stabilization of N- and C-termini to the core domain (middle) and N-terminal localization in cell wall protein (right).

Ielasi et al. 2012; Sim et al. 2013; Veelders et al. 2010). In addition to the PA14 core, key structural features include (i) a calcium-binding loop CBL1, which carries a highly conserved *DcisD* motif that is required for efficient binding of Ca^{2+} ions and mannose ligands, (ii) a CBL2 loop harbouring residues that are conferring ligand binding

without affecting specificity, (iii) a flexible loop L3 that in certain variants can be used to enhance interactions with the mannose ligand, and (iv) a variable loop L2 that forms a Flo-specific subdomain, which is absent in other PA14-type fungal adhesin domains and confers ligand binding specificity, by e.g. discrimination against glucose and galactose

ligands. Biochemical binding studies have revealed that the affinities of FloA-type A domain for mannose are in the low millimolar range, explaining why one-way interactions between carrier and cheater do not lead to efficient floc formation (Veelders et al. 2010). FloA domains are also present in high numbers at the cell surface (Bony et al. 1997), and their high degree of mannosylation, especially along the B-region, leads to efficient homophilic interaction *in vitro* (Goossens et al. 2015). Thus, cell-cell contacts mediated by Flo-type adhesins might be achieved by both heterophilic as well as homophilic interactions and could involve not only protein-glycan, but also glycan-glycan interactions (Goossens et al. 2015).

With respect to structural and functional diversity, it is not yet fully understood why yeast species harbour entire families of very similar Flo-type adhesins that apparently all confer the same flocculation phenotype. In *S. cerevisiae*, four *FLO* genes are able to induce floc formation to comparable degrees (Guo et al. 2000). Here, a recent study suggests that the different *FLO* genes govern selective association of *S. cerevisiae* with specific species of non-*Saccharomyces* yeasts, but the molecular basis for this discrimination is not known (Rossouw et al. 2015). The genome of the yeast *Saccharomycopsis fermentans*, an ascomycete that is distantly related to *S. cerevisiae* and is found in spontaneous wine fermentations, even harbours a family of 34 Flo-type adhesins with PA14-type A domains, whose functions have not been studied so far (Bernardi et al. 2018). Thus, detailed analysis of further Flo-type adhesins and A domains is required, to obtain a more complete picture of their structural and functional diversity.

Epa-type cluster

The Epa-type cluster currently contains 37 PA14-type GPI-CWPs from different *Candida glabrata* strains. *C. glabrata* is a well-known commensal of humans and some other animals, mostly thriving in mucosal tissues including the gut, but gains increasing attention as a pathogen and its surprisingly high genetic diversity (Gabaldon and Fairhead 2019). As an opportunistic human pathogen *C. glabrata* is now known to contain more than 80 GPI-CWP genes that are predominantly located in the subtelomeric regions (Timmermans et al. 2018; Xu et al. 2020). The founding member Epa1 was initially discovered as a fungal adhesin that is required and sufficient to confer binding to human epithelial cells (Cormack et al. 1999). Further comprehensive functional characterization on 17 different *C. glabrata* Epa variants included cell adhesion assays, *in*

vitro binding studies, glycan array screening, single cell atomic force microscopy, mutational analysis and the use of animal infection models. Together, these studies have shown that Epa-type adhesins (i) confer adhesion to human epithelial and macrophage-like cells (Cormack et al. 1999; Kuhn and Vyas 2012), (ii) bind directly to diverse human proteins including mucins, fibronectin and tumour necrosis factor (Ielasi et al. 2016; Ielasi et al. 2014; Zajac et al. 2016), (iii) specifically recognize a wide variety of glycans with terminal α - or β -linked galactosides and non-galactosidic glycans present on human cells and tissues (Diderrich et al. 2015; Ielasi et al. 2016; Maestre-Reyna et al. 2012; Zupancic et al. 2008), (iv) enable efficient binding to clinically relevant plastic materials by conferring strong hydrophobic adhesion forces (El-Kirat-Chatel et al. 2015; Valotteau et al. 2019), and (v) contribute to virulence of *C. glabrata* in diverse murine infection models (Domergue et al. 2005; Vale-Silva et al. 2016). As such, these studies emphasize a key function of Epa-type adhesins in fungal pathogen-host interactions.

Up to date, high-resolution crystal structures of the adhesion domains of *C. glabrata* Epa1 (Ielasi et al. 2012; Maestre-Reyna et al. 2012), Epa6 (Diderrich et al. 2015) and Epa9 (Hoffmann et al. 2020) in complex with diverse glycan ligands have been solved. These studies show that Epa-type domains, in addition to the PA14 core, possess further peripheral structural elements for ligand binding and discrimination. These structural hot spots include two calcium binding loops, CBL1 and CBL2, which form the inner ligand binding pocket, and three further loops, L1-L3, that form the outer pocket. While the surface composition of different EpaA domains is mostly variable, a number of residues within the ligand binding pockets are highly conserved. These include a DcisD motif in CBL1 and an asparagine in CBL2, which confer coordination to a Ca^{2+} ion and together are referred to as the DD-N signature, and a tryptophan residue in loop L3, which confers high affinity ligand binding in the low micromolar range and together with a highly conserved arginine in CBL2 forms the W-R corner signature (Diderrich et al. 2015; Maestre-Reyna et al. 2012). Moreover, *C. glabrata* Epa-type ligand binding pockets carry several highly variable residues at three positions of CBL2 and within the loops L1 and L2. The sequence of the variable positions of CBL2 often correlates with the observed glycan ligand binding patterns of different Epa variants, suggesting that this motif confers ligand binding specificity, a conclusion that is supported by mutational analysis of the Epa1 CBL2 motif (Ielasi et al. 2014; Maestre-Reyna et al. 2012). In certain cases, however, CBL2 composition and ligand binding patterns do not correlate, indicating that other variable elements, e.g.

loops L1 and L2, contribute to ligand recognition (Diderrich et al. 2015). Indeed, a comprehensive structure-based mutational analysis using six different *C. glabrata* Epa paralogs shows that the variable CBL2 loop is key for programming ligand binding specificity and that the variable loop L1 affects host cell binding, but not ligand binding patterns (Hoffmann et al. 2020).

The structural and functional studies on the *C. glabrata* Epa family indicate that variation of specific structural hot spots in the ligand binding pockets of Epa-type domains is a key driver for their diversification and evolution. This view is supported by comparative sequence analysis, which reveals that the DD-N calcium-binding signature is commonly present in 80 % of PA14 domains with conserved DcisD motif (299/376), but that the W-R signature (3%, 13/376) is mostly restricted to variants of the *glabrata* group of *Nakaseomyces* (Diderrich et al. 2015; Gabaldon et al. 2013). Moreover, nine of the functionally well-studied members of *C. glabrata* Epa variants carry unique sequence motifs at the positions II-IV in the variable CBL2 loop, which significantly correlate with glycan binding specificities and are generally not found in the CBL2 regions of the other known Epa-type domains that carry the DD-N and W-R signatures (Diderrich et al. 2015). This supports the view that specific adaptation of CBL2 motifs has evolved after the W-R signatures and significantly contributes to the diverse host specificities observed for different *Nakaseomyces* species (Bolotin-Fukuhara and Fairhead 2014).

Pwp-type cluster

The Pwp-type cluster represents another *C. glabrata* specific group of PA14-type GPI-CWPs. So far, functional analysis has been performed on members of the *C. glabrata* Pwp family consisting of seven paralogs, which in contrast to Epa-type adhesins are mostly located in centromeric regions (de Groot et al. 2008). Whereas deletion of the *PWP7* gene has been reported to result in a significant reduction in adherence to human endothelial cells (Desai et al. 2011), expression of Pwp7 and most other *C. glabrata* Pwp paralogs in an *S. cerevisiae* system does not mediate adhesion to a variety of six different human endothelial or epithelial cells (Hwang-Wong 2016). In addition, a genome-wide expression study using *C. glabrata* axenic cultures and a number of different stress conditions shows that in comparison to housekeeping genes, the general expression levels of *PWP* genes are low and comparable to that of *EPA* genes (Linde et al. 2015). Thus, both the

physiological roles of Pwp-type adhesins and the precise conditions inducing their expression, e.g. in the host, remain to be determined.

No structural or ligand binding studies are available for Pwp-type adhesins so far. Nonetheless, comparative sequence analysis indicates that Pwp-type domains might act as lectins much like other PA14-type adhesion domains, because they contain a PA14 core, CBL1 and CBL2 loops that contain the DD-N signature, and the loops L1 – L3. Like Epa-type members, they lack the Flo-type specific sub-domain in loop L2, but unlike the Epa-type also the W-R signature. Another characteristic of Pwp-type domains is the lack of the CC-motif for disulphide bond formation with the N- and C-termini. Accordingly, this sets Pwp-type adhesins aside from so far studied PA14-adhesins, as they belong to Group II of PA14-dependent adhesins (Figure 2B), which otherwise are GPI-CWPs found in filamentous fungi, some in the family of *Saccharomycodaceae* and a group of *Komagataella* species. Clearly, future structural and functional studies are needed to determine the biochemical functions of Pwp-type domains such as the binding of specific glycan ligands.

Kluyveromyces cluster

As for the Pwp-cluster, no member of the *Kluyveromyces* cluster with its 44 PA14-type GPI-CWPs from 14 species has been structurally or biochemically characterised yet. Given their potential for biotechnology (Spohner et al. 2016) it is interesting to note that *K. lactis* and *K. marxianus* genomes encode for 6 and 8 paralogs, respectively, of PA14-dependent adhesins. All adhesins of *K. marxianus* have a (Y,F)-R signature in the putative carbohydrate binding site and may hence recognize similar ligands as members of the Epa-type. For the probiotic strain *K. marxianus* B0399, adhesion to colonic Caco-2 cells has been observed (Maccaferri et al. 2012), a characteristic that is exerted by some Epa-type adhesins like Epa1, Epa6 and Epa7, but not others (Diderrich et al. 2015; Maestre-Reyna et al. 2012).

PA14-adhesins from Phaffomycetaceae

PA14-type GPI-CWPs from Phaffomycetaceae co-cluster like those from Metschnikowiaceae with PA14-type adhesins from filamentous fungi and are hence distinct from the Saccharomycetal adhesins described so far (Figure 2A). To date, two paralogs originating from the methylotrophic yeast *Komagataella pastoris*, the chitin-end adhesin Cea1/KpFlo1 as the founding member and the orphan lectin

KpFlo2, have been functionally and structurally characterized (Kock et al. 2018). Functional analysis employing high-throughput glycan array screening, *in vitro* glycan binding assays and an *S. cerevisiae* expression system has revealed that Cea1 specifically binds to diverse terminal β -linked N-acetylglucosamines in the low micromolar range *in vitro* and confers efficient binding to non-reducing ends of chitin when presented at the surface of yeast cells. For KpFlo2, no specific glycan binding was found. Thus, Cea1 represents the first fungal cell wall adhesin that strictly recognizes β -capped N-acetylglucosamine of chitinous polymers. It has therefore been proposed that Cea1 might be crucial for interaction of *Komagataella* species with insects (Kock et al. 2018), a hypothesis that is supported by the fact that *K. pastoris* was initially discovered in close association with the fruit fly *Drosophila melanogaster* (Shihata and Mrak 1952).

Crystal structures were solved for Cea1 in complex with N-acetylglucosamine and N,N'-diacetylchitobiose, respectively, and for KpFlo2 (Kock et al. 2018). They reveal that both domains not only consist of the β -sandwich PA14 core, but also contain the loops CBL1, CBL2, L1, L2 and L3. They also contain the DD-N signature present in Flo-type and Epa-type domains, which confers coordination to a Ca^{2+} ion and to the glycan ligands. In contrast, they lack the Flo-type specific subdomain in loop L2 and the Epa-type specific W-R signature. Two unique structural features of Cea1 are its unusually narrow binding site for terminal N-acetylglucosamine moieties and the presence of a lysine residue in loop L3 instead of the tryptophan of the W-R motif that enables a high number of ligand interactions, which explains the low micromolar binding affinity *in vitro*.

In *K. pastoris* and *Komagataella phaffii*, a third paralogous PA14-adhesin, KpFlo3, has been found that shares Ca^{2+} -binding via the DcisD-N motif but that has not been further characterized (Kock et al. 2018). In addition to these Group I PA14-adhesins, Phaffomycetaceae comprise an additional set of Group II PA14-adhesins (Figure 2A, B), which miss not only the CC-motif, but also an intact calcium-binding site due to replacement of the second aspartate of the DcisD-motif by tyrosine. Like other Group II PA14-adhesins, they are mostly located close to the C-terminal ends of cell-wall resident adhesins, but their function is still unknown. Given the lack of typical C-type lectin signatures, the biological role of these Group II PA14-type adhesins may hence clearly differ from *bona fide* carbohydrate-binding adhesins.

Despite their deviations from other saccharomycetal PA14-type adhesin clusters, Cea1-like adhesins share high-affinity ligand binding and the potential for heterotypic cell-cell or cell-substrate interactions with Epa-type

domains. The observation of an orphan lectin like KpFlo2 is reminiscent of many Epa-type adhesins, whose cognate (carbohydrate) ligands could not yet be unambiguously assigned (Diderrich et al. 2015; Kock et al. 2018). Clearly, detailed structure-function relationships of additional *Komagataella* PA14-domains must further define the general properties of this group of putative adhesins.

Further group II PA14-type adhesins

About two third of all putative PA14-type adhesins (375/564) lack the CC-motif required for disulphide-bridged stabilization of the adhesion domain and formation of a rigidified stalk region for connection to subsequent B-region repeats (Figure 2B). Despite their occurrence in many important animal and plant pathogens such as *C. glabrata* or diverse *Fusarium* or *Trichoderma* species, there are no functional or structural data available for this type of cell wall adhesins. Unlike found for Flo-, Epa- or Pwp-type adhesins, 71% (267/375) of these Group II PA14-type adhesion domains are not located at the N-termini of the gene products, but in contrast to the Group II members from Phaffomycetaceae possess a functional DcisD motif (Figure 2B). Accordingly, these cell wall proteins may mediate not only adhesion, but also intra-cell wall cross-linking with endogenous glycans.

Flo11-type adhesins

Fungal adhesins that carry an N-terminal Flo11-type domain (PFAM entry PF10182) are encoded by currently 251 known genes from 86 fungal species, which belong to 9 families in the ascomycetal orders of Saccharomycetales and Schizosaccharomycetales (Figure 3A). The best-characterized Flo11-type adhesin is Flo11 from *S. cerevisiae* (ScFlo11), which was originally discovered as a cell surface flocculin required for substrate adhesion and the formation of invasive filaments on agar surfaces (Lo and Dranginis 1996, 1998). Further genetic studies revealed that ScFlo11 confers the formation of other multicellular growth forms including structured mats on viscous surfaces (Reynolds and Fink 2001), floating flocs on liquid cultures (Fidalgo et al. 2006; Ishigami et al. 2004; Zara et al. 2005), and adhesive biofilms on solid plastic surfaces (Reynolds and Fink 2001). Ultrastructural analysis employing electron microscopy suggests that Flo11 adhesins connect cells by forming an extracellular matrix that involves co-aligned Flo11 fibres (Kraushaar et al. 2015; Vachova et al. 2011). Further biochemical studies with purified ScFlo11A

Figure 3: The class of Flo11-like fungal adhesins.

Left, the SSN of the Flo11 domain protein family (IPR018789, E-value cut-off 10^{-15}) shows that family members are restricted to both the orders of Saccharomycetales and Schizosaccharomycetales. The two structures from *S. cerevisiae* (red) and *K. pastoris* (green) show two surface-exposed aromatic bands (turquoise), which have been claimed to mediate interactions with hydrophobic surfaces as well as with other Flo11-like adhesin domains (right). *Sc*, *Saccharomyces cerevisiae*; *Kp*, *Komagataella pastoris*.

domains and biophysical studies employing AFM-based single cell force spectroscopy have shown that ScFlo11 mediates cell-cell adhesion by homotypic protein-protein interactions (Douglas et al. 2007; Kraushaar et al. 2015), which result in remarkably strong adhesion forces of more than 10 nN between interacting cells that must both express the adhesin (Brückner et al. 2020). These studies have demonstrated a key function of Flo11-type domains in conferring cell-cell and cell-substrate adhesion during multicellular development.

Investigation of *FLO11* adhesin genes in yeasts other than *S. cerevisiae* has not been performed yet. However, expression of a number of Flo11A domains from diverse Saccharomycetales species in an *S. cerevisiae* model has shown that the capacity of these adhesins for conferring cell-cell adhesion through homotypic interactions is highly conserved (Brückner et al. 2020). These studies have further shown that Flo11-type adhesins can act as cell surface receptors, which mediate kin discrimination in mixed *S. cerevisiae* populations that express variants from different species or sub-species (Brückner et al. 2020; Oppler et al. 2019). In addition, Flo11 adhesins confer competitive advantages through cell differentiation in clonal biofilms (Regenberg et al. 2016). Thus, the

physiological functions of Flo11-type adhesins are key not only for the formation of diverse multicellular growth forms that confer environmental protection and promote substrate colonization, but also for preventing the invasion of groups of closely related cooperating individuals by more distantly related species or sub-species.

High-resolution crystal structures of Flo11-type domains have been solved for ScFlo11A (Kraushaar et al. 2015) and for KpFlo11A (Brückner et al. 2020), a variant from the methylotrophic yeast *K. pastoris* of the family of Phaffomycetaceae. Although the two variants share only 32% identity, their overall structures show a high degree of similarity. They both consist of a fibronectin type III (FN3) core, a fold that forms a large family within the immunoglobulin (Ig) superfamily. In addition, both orthologs expose an unusually large number of conserved aromatic residues at the protein surface, which are arranged in two bands that have a similar but not identical configuration. Systematic mutational analysis of surface-exposed aromatic residues of ScFlo11A has shown that the aromatic bands are essential for efficient protein-protein interactions *in vitro* as well as for cell-cell adhesion at single cell level and in populations *in vivo* (Brückner et al. 2020)(Figure 3B). Comparison of homotypic and

heterotypic interactions of ScFlo11 and KpFlo11 further revealed that heterotypic protein-protein binding as well as cell-cell adhesion forces are significantly reduced when compared to the respective homotypic interactions. It has therefore been proposed that discrimination between Flo11-type domains from different yeast species at the molecular level might be conferred by inefficient interaction of their differentially arranged aromatic bands.

Comparative analysis of 52 Flo11A domains from different *S. cerevisiae* strains has revealed the presence of a small insert of about 15 amino acids in a number of variants, that are exclusively found in the 'sake' lineage of non-mosaic strains (Brückner et al. 2020; Liti et al. 2009). Remarkably, this insert is sufficient to confer sub-species discrimination at single cell level and in competing *S. cerevisiae* populations and might have originated from a single genetic event. As such, Flo11-type domains have become valuable models for investigating how the structure of microbial cell surface adhesins can evolve to permit diversification at species and sub-species levels without compromising their core functions.

Als-type adhesins

Fungal adhesins carrying an N-terminal agglutinin-like sequence (Als) region (Pfam entry PF11766) are encoded by currently 251 known genes from 83 fungal species, which are restricted to the ascomycetal classes of Saccharomycetes (74) and a few Sordariomycetes (9). This type of adhesins (Figure 4A) is a minor part of the very large adhesion domain superfamily (INTERPRO entry IPR008966) with its huge variety of bacterial and eukaryotic lectins, collagen-binding proteins and fimbrial adhesins.

The functionally best-studied adhesins in this group are found in the most frequently isolated human fungal pathogen *C. albicans*, which contains the Als family encompassing eight members (Als1 to Als7, and Als9) (Hoyer 2001). The founding member *ALS1* was initially discovered as a gene that is induced during hyphal development and encodes a protein with similarity to the *S. cerevisiae* mating alpha-agglutinin (SAG1) that belongs to the family of Als-type adhesins (SAG1 group, Figure 4A) as well and confers cell type-specific cell-cell adhesion (Hoyer et al. 1995). Since then, a plethora of functions have been described for Als family members, predominantly for Als1 and Als3, which have been reviewed in great detail (Hoyer and Cota 2016). Among others, these functions include (i) adhesion to human endothelial and epithelial cells, (ii) binding to fibronectin, fibrinogen, laminin, collagen, cadherins and further human proteins with affinities in the low micromolar to

millimolar range, (iii) binding to human glycans containing fucose or N-acetyl-glucosamine with micromolar affinities, (iv) adherence to diverse abiotic plastic and glass surfaces, and (v) enhancement of *C. albicans* virulence in animal infection models. These functions, much like that of *C. glabrata* Epa adhesins, emphasize the key role of *C. albicans* Als proteins in host-pathogen interactions. In addition, Als1 and Als3 confer binding to bacterial pathogens such as *Streptococcus gordonii* and *Staphylococcus aureus*, indicating a role during the formation of clinically relevant microbial communities (Peters et al. 2012; Silverman et al. 2010).

High resolution structures are available for the N-terminal adhesion domains of Als9 in complex with a peptide from the C-terminal end of human fibrinogen gamma (Salgado et al. 2011) and for Als3 in complex with hepta-threonine (Lin et al. 2014). These structures provide important functional insights and show that Als domains comprise two tandem immunoglobulin G (IgG) domains (N1 and N2), which resemble MSCRAMM domains found in fibrinogen-binding adhesins from the bacterial pathogens *S. aureus* and SdrG from *Staphylococcus epidermidis*. The region between N1 and N2 forms a peptide-binding cavity (PBC), which buries up to six C-terminal residues of polypeptides and enables high-affinity ligand binding by establishing a salt-bridge between an invariant lysine at the end of the PBC and the C-terminal carboxylate of variable peptide ligands. The PBC further interacts with the backbone of peptides by forming several protein-peptide interactions through hydrogen bonding and a variable network of water molecules that contribute to the recognition of a broad range of diverse peptide sequences. The structural data available for Als-type domains so far clearly support a main function as peptide binding adhesins (Cota and Hoyer 2015). Whether Als-type domains can also act as lectins, which mediate specific binding to glycan ligands, is currently not supported by structural analysis.

Als-type fungal adhesins have also been studied with respect to conferring homotypic interactions. Purified *C. albicans* Als-type domains consisting of IgG domains N1 and N2 are unable to bind to each other with high affinity (Lin et al. 2014), as has been found for Flo11-type domains. In full-length Als proteins, however, Als-type domains are neighboured by amyloid forming regions (AFR), which have been demonstrated to confer homotypic interactions (Lipke et al. 2012). Moreover, AFR regions can interact with the N1 domain in a peptide ligand-dependent manner, which prevents homotypic AFR interactions. It has therefore been proposed that in the absence of suitable ligands, Als adhesins are able to confer adhesion between *C. albicans* cells though amyloid formation of AFRs. This mechanism could enable *C. albicans* to form multicellular aggregates such as

Figure 4: The class of Als-like fungal adhesins.

(A) The Als-like fungal adhesins are part of the very large adhesin domain superfamily (ADS; IPR008966, SSN shown as inlay with E-value cut-off 10^{-15}), which covers all domains of life. The two Ig-domains of Als proteins from *Candida albicans* (Ca) bind ligands along the interface region (B) The Venn-diagram shows the co-occurrence of Als-, Flo11- and PA14-like adhesin domains in 255 fungal species as based on analysis of the corresponding INTERPRO families IPR08966, IPR018789 and IPR037524. The numbers in parentheses correspond to the number of species found for each adhesin family.

protective biofilms that are less susceptible to host defence or antifungal drugs (Lin et al. 2014).

Detailed functional analysis of Als-type adhesins from species other than *C. albicans* has not been performed yet, but comparative genomics reveals the presence of numerous *ALS* genes in the *Candida parapsilosis* complex including the closely related *C. parapsilosis*, *Candida orthopsilosis* and *Candida metapsilosis* species (Lombardi et al. 2019; Oh et al. 2019). While the predicted Als proteins harbour most common features of the *C. albicans* Als family, including the presence of PBC and AFRs in the N-terminal regions, they also have certain unique features. Molecular modeling, for instance, reveals large differences in shape and charge of different PBCs, suggesting functional diversity with regard to the preferred peptide ligands. Clearly, future biochemical and structural analysis of a wide variety of diverse Als-type domains will contribute to a deeper understanding of their molecular and functional evolution.

Occurrences and other potential fungal adhesion domains

Of the three major GPI-adhesin types, which have been functionally and structurally characterized in detail, the PA14-type sticks out due to its wide occurrence in Ascomycetes. Unlike the other two types, i.e. Flo11- and Als-

type, PA14-type adhesins occur in about three quarters of ascomycetal species without accompanying Flo11- or Als-adhesins (Figure 4B). However, this does not necessarily indicate that the largest determinant of fungal adhesion is binding to glycan structures of host surfaces. For example, Group II PA-14 adhesins lack key features required for C-type lectin function. Additionally, many Group I PA-14 adhesins with intact DcisD motif characterized so far had to be assigned as orphan lectins given the lack of detectable ligands. The delineation of the binding specificities of these adhesins may be highly relevant to understand fungal biology, e.g. in terms of host specificity. In contrast, Flo11-type adhesins as well as Als-type adhesins apparently provide additional, but not necessarily essential adherence capabilities to fungal species, because they are associated with other adhesin types in the case of 81 and 75%, respectively, of ascomycetal species (Figure 4B). Moreover, Flo11 has been claimed to act like a cell-wall tethered hydrophobin (Kraushaar et al. 2015) for mediating interactions to hydrophobic surfaces including those of other yeast cells, rather than a ligand-specific adhesin, whereas the Als-type apparently exhibits considerable specificity towards C-terminal peptide ends.

Several putative domains other than the three major GPI-adhesin types have been found to mediate adhesive function in several fungal species. De Groot et al. discovered additional adhesin-like wall proteins (Awps) in *C. glabrata* CBS138, besides its seven Pwp paralogs (de

Groot et al. 2013; de Groot et al. 2008), which currently amount to 13 members. However, the N-terminal domains of Awp proteins themselves belong to different protein families. Awp1 and Awp3 are for instance related to fungal haze-protective-factor proteins, whereas the other Awp members belong to at least three different subfamilies (de Groot et al. 2013). Interestingly, these adhesin candidates are found among cell proteins that are up-regulated during host colonization by clinical *C. glabrata* strains on proteome, transcriptome and genome levels (Carrete et al. 2019; Gomez-Molero et al. 2015; Kraneveld et al. 2011). Another example is the MAD1-type of adhesins, which utilize cysteine-rich fungal extracellular module (CFEM) domains (Li et al. 2016; Wang and St Leger 2007) and apparently contribute to fungal adhesion to nematodes and insects. These and many other GPI-CWP with assigned adhesion function await further biochemical, functional and structural studies to understand their role in controlling different fungal life styles.

GPI-CWP adhesins – to be sorted or not to be sorted into the cell wall?

One unresolved issue in the context of GPI-anchored adhesins is how sorting into the cell wall occurs, as all GPI-anchored proteins initially end up at the plasma membrane (Figure 5). Some of these proteins remain there as GPI-plasma membrane proteins (GPI-PMPs), whereas others such as adhesins are transferred onto cell wall glucans as GPI-CWPs. This division is not absolutely strict, as several GPI-anchored proteins can end up in both locations (Pittet and Conzelmann 2007). In *S. cerevisiae*, the two paralogous transglycosidases, Dfg5 and Dcw1, which mediate membrane-to-cell wall transfer of GPI-CWPs, are themselves produced as GPI-PMPs. Here, their GPI anchorage is sufficiently stable to maintain their membrane residence for allowing Dfg5-catalyzed transfer of GPI-CWPs to cell wall glycans.

In yeast, temperature-sensitive mutants of the cell-division cycle gene *CDC1* were shown to stop growth in early G2 phase with small buds and replicated DNA, a phenomenon that was also found in mutants of *DCW1* (Kitagaki et al. 2002, 2004). Cdc1 is a phosphodiesterase that removes the EtN-P added to mannose-1 of the GPI core glycan by Mcd4 during GPI-anchor biosynthesis and can be deleted in a *mcd4Δ* background without loss of viability (Vazquez et al. 2014). Another phosphodiesterase, Ted1, removes the EtN-P from mannose-2, and acts together with Emp24 and Erv25 at the ER exit sites (ERES) for facilitating efficient cargo exit (Haass et al. 2007). These modifications

of the GPI-core glycan may disrupt binding of the GPI-anchor by Dfg5 enzymes, thus preventing GPI-CWP transfer onto cell wall glycans. This hypothesis explains the comparable phenotypes of *cdc1* and *dfg5 dcw1* mutants, which both exhibit growth arrest and a compromised lipid-to-wall transfer of GPI-CWPs.

In contrast to Cdc1, removal of EtN-P from mannose-2 by Ted1 is not essential, at least in *S. cerevisiae*, though its deletion delays ER-export of GPI-anchored proteins due to its interaction with the p24-complex during COPII trafficking (Manzano-Lopez et al. 2015). *TED1* deletion mutants hence resemble deletion mutants of the p24-complex component encoding genes *EMP24* and *ERV25*, which both show the same ER-exit defects (Belden and Barlowe 1996; Schimmoller et al. 1995). The consequences for membrane-to-cell wall transfer by Dfg5 enzymes in *ted1Δ* cells is hence not as fatal as that of the *cdc1Δ* mutants, because the 6-hydroxy group of mannose-2 in GPI-anchors may not be recognized by these enzymes. Accordingly, *ted1Δ* mutants exert a growth behaviour in rich medium as observed for the wild type (Yoko et al. 2018).

Cwh43 mediates a key step of GPI-lipid remodelling, namely transfer of the GPI-anchored protein precursor from phosphatidylinositol (PI) to inositol-phosphorylceramide (IPC). Both EtN-PS of mannose-1 and -2 are required for ceramide-remodelling by Cwh43 (Benachour et al. 1999; Zhu et al. 2006). While the GPI-CWP Cwp2 possesses a PI in wild type cells, GPI-PMPs such as Gas1 harbours an IPC lipid. In *cwh43Δ* mutants, Gas1 is anchored instead by PI and preferably localized to the cell wall. In yeast, two models have been proposed to explain when and how Cwh43 acts on lipid remodelling. The sequential model suggests a main pathway, where Cwh43 utilizes a PI-containing diacylglycerol after Gup1. The divergent model proposes an alternative route utilizing lysoPI for ceramide remodelling and thereby separating the reactions of Gip1 and Cwh43 (Ghugtyal et al. 2007; Umemura et al. 2003). However, at least in the filamentous fungus *Aspergillus fumigatus* such an alternative route could be excluded (Li et al. 2018).

Apparently, the fate of GPI-CWPs and GPI-PMPs is already encoded during the maturation of the GPI-anchor in the secretory pathway, while Dfg5 enzymes decode the previous decision at the plasma membrane (Vogt et al. 2020). A sequence of events can be suggested in the ER of yeasts (and probably other fungi possessing two paralogs of the mammalian PGAP5, i.e. Cdc1 and Ted1), upon which the destination of GPI-anchored proteins is determined (Figure 5). Initially remodelling of the lipid occurs in the first three steps of the sequential pathway by Bst1, Per1,

Figure 5: Ethanolamine-phosphate modifications control GPI-based cell wall-incorporation by Dfg5-enzymes. After GPI-precursor biosynthesis, the GPI-transamidase complex transfers target proteins onto the EtN-P of mannose-3, upon which Bst1, Per1, and Gup1 remodel the GPI-lipids. Depending on the destination, either Cdc1 removes the EtN-P from mannose-1, or Cwh43 catalyses lipid exchange from PI to IPC. Thereby, the EtN-P code for the final destination of GPI-anchored proteins, i.e. GPI-CWP vs. GPI-PMP, is generated. Subsequently, Ted1 removes EtN-P from mannose-2 to facilitate ER-exit. After further processing in the Golgi, Dfg5-transamidases decode the presence of EtN-P modifications within the GPI-core and select the correctly processed subset of GPI-anchored proteins for final maturation as GPI-CWPs.

and Gup1 directly after the GPI-transamidase complex adds the target protein to the GPI-precursor (Kinoshita and Fujita 2016). GPI-lipid remodelling, but not GPI-glycan remodelling, is the crucial step for concentration and pre-sorting at ERESs (Manzano-Lopez et al. 2015). The next step determines the destination, because designated GPI-CWPs are recognized by Cdc1 (Figure 5, upper route), which not only removes the EtN-P at Man1, but also prevents ceramide transfer initiated by Cwh43 (Vazquez et al. 2014). Subsequently, Ted1 removes EtN-P from mannose-2, which results in the mature ER-form of the GPI-glycan and facilitates efficient COPII-vesicular traffic to the Golgi as initiated by the p24-complex (Haass et al. 2007; Manzano-Lopez et al. 2015). Upon export to the plasma membrane, the glycan of GPI-anchored proteins containing a PI-lipid are recognized by Dfg5-proteins and further transferred to cell wall glycans. GPI-PMPs, however, are recognized by Cwh43 (Figure 5, lower route), which remodels the lipid from PI to IPC. As the preferred substrate of Cdc1 are GPI-anchors with a diacylglycerol lipid this step is skipped and the GPI-ceramide is directly handed over to Ted1 (Vazquez et al. 2014) for removal of the EtN-P from mannose-2 and p24-mediated cargo export to the Golgi. This results in a secreted GPI-anchored protein possessing an IPC and an EtN-P at mannose-1. This modification prohibits recognition of Dfg5-enzymes and leaves GPI-PMP in the plasma membrane (Vogt et al. 2020). As animals lack GPI-CWPs, this might also explain why they possess a single PGAP5-homolog for GPI-glycan processing for ensuring efficient ER to Golgi transport by removing EtN-P from mannose-2, but leaving EtN-P at mannose-1.

Conclusions

As outlined before, our understanding of fungal adhesins is still fragmentary despite the increasing progress in understanding major families like the PA14-type adhesins. This includes the assignment of ligand specificities as well as physiological roles. As learnt from Flo11-type adhesins, this does not necessarily imply highly specific molecular cues, but may include more general biophysical properties such as hydrophobicity and charge.

The control on fungal life style that is exerted by GPI-adhesins makes these proteins attractive, but difficult to handle, targets for controlling plant and human pathogens, which are continuously arising (Almeida et al. 2019). For example, *Candida* spp., *Aspergillus* spp., and *Cryptococcus* spp. are increasing threats for immunosuppressed patients. Currently, an increase in resistance to available therapeutic antimycotics can be observed. Echinocandin-class drugs, for instance, effectively target cell wall biosynthesis by inhibiting the β 1,3-glucan synthases. However, while echinocandins are relatively well tolerated in patients, excessive use of this class of antimycotics led to an increase of echinocandin-resistant fungi, especially of *Candida* spp. One route to combat fungal infections might be the development of anti-adhesive compounds, but this might be an unavailing effort given the plethora of fungal adhesins with their widely diverging binding specificities. We therefore propose that the development of antimycotics that target the maturation pathway of GPI-anchored adhesins might be a more promising approach, because this pathway forms a bottleneck that is highly conserved in all fungi.

Acknowledgments: This work was supported by the Collaborative Research Consortium 987 (SFB 987) of the Deutsche Forschungsgemeinschaft (DFG).

Author contribution: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: None declared.

Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

- Almeida, F., Rodrigues, M.L., and Coelho, C. (2019). The still underestimated problem of fungal diseases worldwide. *Front. Microbiol.* 10: 214.
- Ao, J., Chinnici, J.L., Maddi, A., and Free, S.J. (2015). The N-linked outer chain mannans and the Dfg5p and Dcw1p endo- α -1,6-mannanases are needed for incorporation of *Candida albicans* glycoproteins into the cell wall. *Eukaryot. Cell* 14: 792–803.
- Bauer, F.F., Govender, P., and Bester, M.C. (2010). Yeast flocculation and its biotechnological relevance. *Appl. Microbiol. Biotechnol.* 88: 31–39.
- Belden, W.J. and Barlowe, C. (1996). Erv25p, a component of COPII-coated vesicles, forms a complex with Emp24p that is required for efficient endoplasmic reticulum to Golgi transport. *J. Biol. Chem.* 271: 26939–26946.
- Benachour, A., Sipos, G., Flury, I., Reggiori, F., Canivenc-Gansel, E., Vionnet, C., Conzelmann, A., and Benghezal, M. (1999). Deletion of GPI7, a yeast gene required for addition of a side chain to the glycosylphosphatidylinositol (GPI) core structure, affects GPI protein transport, remodeling, and cell wall integrity. *J. Biol. Chem.* 274: 15251–15261.
- Bernardi, B., Kayacan, Y., and Wendland, J. (2018). Expansion of a telomeric FLO/ALS-like sequence gene family in *Saccharomycopsis fermentans*. *Front. Genet.* 9: 536.
- Bolotin-Fukuhara, M. and Fairhead, C. (2014). *Candida glabrata*: a deadly companion?. *Yeast* 31: 279–288.
- Bony, M., Thines-Sempoux, D., Barre, P., and Blondin, B. (1997). Localization and cell surface anchoring of the *Saccharomyces cerevisiae* flocculation protein Flo1p. *J. Bacteriol.* 179: 4929–4936.
- Bowman, S.M. and Free, S.J. (2006). The structure and synthesis of the fungal cell wall. *Bioessays* 28: 799–808.
- Brown, G.D., Denning, D.W., Gow, N.A., Levitz, S.M., Netea, M.G., and White, T.C. (2012). Hidden killers: human fungal infections. *Sci. Transl. Med.* 4: 165rv113.
- Brückner, S. and Mösch, H.U. (2012). Choosing the right lifestyle: adhesion and development in *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* 36: 25–58.
- Brückner, S., Schubert, R., Kraushaar, T., Hartmann, R., Hoffmann, D., Jelli, E., Drescher, K., Muller, D.J., Oliver Essen, L.O., and Mösch, H.U. (2020). Kin discrimination in social yeast is mediated by cell surface receptors of the Flo11 adhesin family. *eLife* 9: e55587.
- Carrete, L., Ksiezopolska, E., Gomez-Molero, E., Angoulvant, A., Bader, O., Fairhead, C., and Gabaldon, T. (2019). Genome comparisons of *Candida glabrata* serial clinical isolates reveal patterns of genetic variation in infecting clonal populations. *Front. Microbiol.* 10: 112.
- Cormack, B.P., Ghorri, N., and Falkow, S. (1999). An adhesin of the yeast pathogen *Candida glabrata* mediating adherence to human epithelial cells. *Science* 285: 578–582.
- Cota, E. and Hoyer, L.L. (2015). The *Candida albicans* agglutinin-like sequence family of adhesins: functional insights gained from structural analysis. *Future Microbiol.* 10: 1635–1548.
- Dawkins, R. (1976). *The selfish gene*. Oxford University Press: Oxford.
- de Groot, P.W., Bader, O., de Boer, A.D., Weig, M., and Chauhan, N. (2013). Adhesins in human fungal pathogens: glue with plenty of stick. *Eukaryot. Cell* 12: 470–481.
- de Groot, P.W., Hellingwerf, K.J., and Klis, F.M. (2003). Genome-wide identification of fungal GPI proteins. *Yeast* 20: 781–796.
- de Groot, P.W., Kraneveld, E.A., Yin, Q.Y., Dekker, H.L., Gross, U., Crielgaard, W., de Koster, C.G., Bader, O., Klis, F.M., and Weig, M. (2008). The cell wall of the human pathogen *Candida glabrata*: differential incorporation of novel adhesin-like wall proteins. *Eukaryot. Cell* 7: 1951–1964.
- Desai, C., Mavrianos, J., and Chauhan, N. (2011). *Candida glabrata* Pwp7p and Aed1p are required for adherence to human endothelial cells. *FEMS Yeast Res.* 11: 595–601.
- Diderrich, R., Kock, M., Maestre-Reyna, M., Keller, P., Steuber, H., Rupp, S., Essen, L.O., and Mösch, H.U. (2015). Structural hot spots determine functional diversity of the *Candida glabrata* epithelial adhesin family. *J. Biol. Chem.* 290: 19597–19613.
- Domergue, R., Castano, I., De Las Penas, A., Zupancic, M., Lockatell, V., Hebel, J.R., Johnson, D., and Cormack, B.P. (2005). Nicotinic acid limitation regulates silencing of *Candida* adhesins during UTI. *Science* 308: 866–870.
- Douglas, L.M., Li, L., Yang, Y., and Dranginis, A.M. (2007). Expression and characterization of the flocculin Flo11/Muc1, a *Saccharomyces cerevisiae* mannoproteins with homotypic properties of adhesion. *Eukaryot. Cell* 6: 2214–2221.
- Dranginis, A.M., Rauceo, J.M., Coronado, J.E., and Lipke, P.N. (2007). A biochemical guide to yeast adhesins: glycoproteins for social and antisocial occasions. *Microbiol. Mol. Biol. Rev.* 71: 282–294.
- El-Kirat-Chatel, S., Beaussart, A., Derclaye, S., Alsteens, D., Kucharikova, S., Van Dijck, P., and Dufrene, Y.F. (2015). Force nanoscopy of hydrophobic interactions in the fungal pathogen *Candida glabrata*. *ACS Nano* 9: 1648–1655.
- Fidalgo, M., Barrales, R.R., Ibeas, J.I., and Jimenez, J. (2006). Adaptive evolution by mutations in the *FLO11* gene. *Proc. Natl. Acad. Sci. U.S.A.* 103: 11228–11233.
- Fisher, R.M. and Regenber, B. (2019). Multicellular group formation in *Saccharomyces cerevisiae*. *Proc. Biol. Sci.* 286: 20191098.
- Frieman, M.B. and Cormack, B.P. (2003). The omega-site sequence of glycosylphosphatidylinositol-anchored proteins in *Saccharomyces cerevisiae* can determine distribution between the membrane and the cell wall. *Mol. Microbiol.* 50: 883–896.
- Frieman, M.B., McCaffery, J.M., and Cormack, B.P. (2002). Modular domain structure in the *Candida glabrata* adhesin Epa1p, a beta1,6 glucan-cross-linked cell wall protein. *Mol. Microbiol.* 46: 479–492.
- Gabaldon, T. and Fairhead, C. (2019). Genomes shed light on the secret life of *Candida glabrata*: not so asexual, not so commensal. *Curr. Genet.* 65: 93–98.
- Gabaldon, T., Martin, T., Marcet-Houben, M., Durrens, P., Bolotin-Fukuhara, M., Lespinet, O., Arnaise, S., Boissard, S., Aguilera, G., Atanasova, R., et al. (2013). Comparative genomics of emerging pathogens in the *Candida glabrata* clade. *BMC Genom.* 14: 623.

- Garcia-Rubio, R., de Oliveira, H.C., Rivera, J., and Trevijano-Contador, N. (2019). The fungal cell wall: *Candida*, *Cryptococcus*, and *Aspergillus* species. *Front. Microbiol.* 10: 2993.
- Gerlt, J.A., Bouvier, J.T., Davidson, D.B., Imker, H.J., Sadkhin, B., Slater, D.R., and Whalen, K.L. (2015). Enzyme Function Initiative-Enzyme Similarity Tool (EFI-EST): a web tool for generating protein sequence similarity networks. *Biochim. Biophys. Acta* 1854: 1019–1037.
- Ghugtyal, V., Vionnet, C., Roubaty, C., and Conzelmann, A. (2007). CWH43 is required for the introduction of ceramides into GPI anchors in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 65: 1493–1502.
- Gomez-Molero, E., de Boer, A.D., Dekker, H.L., Moreno-Martinez, A., Kraneveld, E.A., Ichsan, Chauhan, N., Weig, M., de Soet, J.J., de Koster, C.G., Bader, O., et al. (2015). Proteomic analysis of hyperadhesive *Candida glabrata* clinical isolates reveals a core wall proteome and differential incorporation of adhesins. *FEMS Yeast Res.* 15, <https://doi.org/10.1093/femsyr/fov098>.
- Goossens, K.V., Ielasi, F.S., Nookaew, I., Stals, I., Alonso-Sarduy, L., Daenen, L., Van Mulders, S.E., Stassen, C., van Eijdsden, R.G., Siewers, V., et al. (2015). Molecular mechanism of flocculation self-recognition in yeast and its role in mating and survival. *mBio* 6, <https://doi.org/10.1128/mbio.00427-15>.
- Guo, B., Styles, C.A., Feng, Q., and Fink, G.R. (2000). A *Saccharomyces* gene family involved in invasive growth, cell-cell adhesion, and mating. *Proc. Natl. Acad. Sci. U.S.A.* 97: 12158–12163.
- Gow, N.A.R., Latge, J.P., and Munro, C.A. (2017). The fungal cell wall: structure, biosynthesis, and function. *Microbiol. Spectr.* 5, <https://doi.org/10.1128/microbiolspec.FUNK-0035-2016>.
- Haass, F.A., Jonikas, M., Walter, P., Weissman, J.S., Jan, Y.N., Jan, L.Y., and Schuldiner, M. (2007). Identification of yeast proteins necessary for cell-surface function of a potassium channel. *Proc. Natl. Acad. Sci. U.S.A.* 104: 18079–18084.
- Hamilton, W.D. (1964). The genetical evolution of social behaviour. II. *J. Theor. Biol.* 7: 17–52.
- Hoffmann, D., Diderrich, R., Reithofer, V., Friederichs, S., Kock, M., Essen, L.-O., and Mösch, H.-U. (2020). Functional reprogramming of *Candida glabrata* epithelial adhesins: the role of conserved and variable structural motifs in ligand binding. *J. Biol. Chem.* 295: 12512–12524.
- Hoyer, L.L. (2001). The ALS gene family of *Candida albicans*. *Trends Microbiol.* 9: 176–180.
- Hoyer, L.L. and Cota, E. (2016). *Candida albicans* agglutinin-like sequence (Als) family vignettes: a review of als protein structure and function. *Front. Microbiol.* 7: 280.
- Hoyer, L.L., Scherer, S., Shatzman, A.R., and Livi, G.P. (1995). *Candida albicans* ALS1: domains related to a *Saccharomyces cerevisiae* sexual agglutinin separated by a repeating motif. *Mol. Microbiol.* 15: 39–54.
- Huang, G., Zhang, M., and Erdman, S.E. (2003). Posttranslational modifications required for cell surface localization and function of the fungal adhesin Aga1p. *Eukaryot. Cell* 2: 1099–1114.
- Hwang-Wong, E. (2016). *Two early processes during infection by the fungal pathogen Candida glabrata: adherence and alkanization*. Johns Hopkins University, Baltimore, Maryland, pp. 185.
- Ielasi, F.S., Alioscha-Perez, M., Donohue, D., Claes, S., Sahli, H., Schols, D., and Willaert, R.G. (2016). Lectin-glycan interaction network-based identification of host receptors of microbial pathogenic adhesins. *mBio* 7, <https://doi.org/10.1128/mbio.00584-16>.
- Ielasi, F.S., Decanniere, K., and Willaert, R.G. (2012). The epithelial adhesin 1 (Epa1p) from the human-pathogenic yeast *Candida glabrata*: structural and functional study of the carbohydrate-binding domain. *Acta Crystallogr D Biol Crystallogr* 68: 210–217.
- Ielasi, F.S., Verhaeghe, T., Desmet, T., and Willaert, R.G. (2014). Engineering the carbohydrate-binding site of Epa1p from *Candida glabrata*: generation of adhesin mutants with different carbohydrate specificity. *Glycobiology* 24: 1312–1322.
- Ishigami, M., Nakagawa, Y., Hayakawa, M., and Imura, Y. (2004). FLO11 is essential for flor formation caused by the C-terminal deletion of NRG1 in *Saccharomyces cerevisiae*. *FEMS Microbiol. Lett.* 237: 425–430.
- Kinoshita, T. and Fujita, M. (2016). Biosynthesis of GPI-anchored proteins: special emphasis on GPI lipid remodeling. *J. Lipid Res.* 57: 6–24.
- Kitagaki, H., Ito, K., and Shimoi, H. (2004). A temperature-sensitive dcw1 mutant of *Saccharomyces cerevisiae* is cell cycle arrested with small buds which have aberrant cell walls. *Eukaryot. Cell* 3: 1297–1306.
- Kitagaki, H., Wu, H., Shimoi, H., and Ito, K. (2002). Two homologous genes, DCW1 (YKL046c) and DFG5, are essential for cell growth and encode glycosylphosphatidylinositol (GPI)-anchored membrane proteins required for cell wall biogenesis in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 46: 1011–1022.
- Kock, M., Brückner, S., Wozniak, N., Maestre-Reyna, M., Veelders, M., Schlereth, J., Mösch, H.U., and Essen, L.O. (2018). Structural and functional characterization of PA14/Flo5-like adhesins from *Komagataella pastoris*. *Front. Microbiol.* 9: 2581.
- Komath, S.S., Singh, S.L., Pratyusha, V.A., and Sah, S.K. (2018). Generating anchors only to lose them: the unusual story of glycosylphosphatidylinositol anchor biosynthesis and remodeling in yeast and fungi. *IUBMB Life* 70: 355–383.
- Kraneveld, E.A., de Soet, J.J., Deng, D.M., Dekker, H.L., de Koster, C.G., Klis, F.M., Crielaard, W., and de Groot, P.W. (2011). Identification and differential gene expression of adhesin-like wall proteins in *Candida glabrata* biofilms. *Mycopathologia* 172: 415–427.
- Kraushaar, T., Brückner, S., Veelders, M., Rhinow, D., Schreiner, F., Birke, R., Pagenstecher, A., Mösch, H.U., and Essen, L.O. (2015). Interactions by the fungal Flo11 adhesin depend on a fibronectin type III-like adhesin domain girdled by aromatic bands. *Structure* 23: 1005–1017.
- Kuhn, D.M. and Vyas, V.K. (2012). The *Candida glabrata* adhesin Epa1p causes adhesion, phagocytosis, and cytokine secretion by innate immune cells. *FEMS Yeast Res.* 12: 398–414.
- Lesage, G. and Bussey, H. (2006). Cell wall assembly in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 70: 317–343.
- Li, J., Liu, Y., Zhu, H., and Zhang, K.Q. (2016). Phylogenetic analysis of adhesion related genes Mad1 revealed a positive selection for the evolution of trapping devices of nematode-trapping fungi. *Sci. Rep.* 6: 22609.
- Li, J., Mouyna, I., Henry, C., Moyrand, F., Malosse, C., Chamot-Rooke, J., Janbon, G., Latge, J.P., and Fontaine, T. (2018). Glycosylphosphatidylinositol anchors from galactomannan and GPI-anchored protein are synthesized by distinct pathways in *Aspergillus fumigatus*. *J Fungi (Basel)* 4, <https://doi.org/10.3390/jof4010019>.
- Lin, J., Oh, S.H., Jones, R., Garnett, J.A., Salgado, P.S., Rusnakova, S., Matthews, S.J., Hoyer, L.L., and Cota, E. (2014). The peptide-

- binding cavity is essential for Als3-mediated adhesion of *Candida albicans* to human cells. *J. Biol. Chem.* 289: 18401–18412.
- Linde, J., Duggan, S., Weber, M., Horn, F., Sieber, P., Hellwig, D., Riege, K., Marz, M., Martin, R., Guthke, R., et al. (2015). Defining the transcriptomic landscape of *Candida glabrata* by RNA-Seq. *Nucleic Acids Res.* 43: 1392–1406.
- Lipke, P.N. (2018). What we do not know about fungal cell adhesion molecules. *J Fungi (Basel)* 4: 59.
- Lipke, P.N., Garcia, M.C., Alsteens, D., Ramsook, C.B., Klotz, S.A., and Dufrene, Y.F. (2012). Strengthening relationships: amyloids create adhesion nanodomains in yeasts. *Trends Microbiol.* 20: 59–65.
- Lipke, P.N., Klotz, S.A., Dufrene, Y.F., Jackson, D.N., and Garcia-Sherman, M.C. (2018). Amyloid-like β -aggregates as force-sensitive switches in fungal biofilms and infections. *Microbiol. Mol. Biol. Rev.* 82, <https://doi.org/10.1128/MMBR.00035-17>.
- Liti, G., Carter, D.M., Moses, A.M., Warringer, J., Parts, L., James, S.A., Davey, R.P., Roberts, I.N., Burt, A., Koufopanou, V., et al. (2009). Population genomics of domestic and wild yeasts. *Nature* 458: 337–341.
- Lo, W.S. and Dranginis, A.M. (1998). The cell surface flocculin Flo11 is required for pseudohyphae formation and invasion by *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 9: 161–171.
- Lo, W.S. and Dranginis, A.M. (1996). *FLO11*, a yeast gene related to the *STA* genes, encodes a novel cell surface flocculin. *J. Bacteriol.* 178: 7144–7151.
- Lombardi, L., Zoppo, M., Rizzato, C., Bottai, D., Hernandez, A.G., Hoyer, L.L., and Tavanti, A. (2019). Characterization of the *Candida orthopsilosis* agglutinin-like sequence (ALS) genes. *PLoS One* 14: e0215912.
- Lu, C.F., Montijn, R.C., Brown, J.L., Klis, F., Kurjan, J., Bussey, H., and Lipke, P.N. (1995). Glycosyl phosphatidylinositol-dependent cross-linking of alpha-agglutinin and β 1,6-glucan in the *Saccharomyces cerevisiae* cell wall. *J. Cell Biol.* 128: 333–340.
- Maccaferri, S., Klinder, A., Brigidi, P., Cavina, P., and Costabile, A. (2012). Potential probiotic *Kluyveromyces marxianus* B0399 modulates the immune response in Caco-2 cells and peripheral blood mononuclear cells and impacts the human gut microbiota in an in vitro colonic model system. *Appl. Environ. Microbiol.* 78: 956–964.
- Maddi, A., Fu, C., and Free, S.J. (2012). The *Neurospora crassa* dfg5 and dcw1 genes encode alpha-1,6-mannanases that function in the incorporation of glycoproteins into the cell wall. *PLoS One* 7: e38872.
- Maestre-Reyna, M., Diderrich, R., Veelders, M.S., Eulenburg, G., Kalugin, V., Brückner, S., Keller, P., Rupp, S., Mösch, H.U., and Essen, L.O. (2012). Structural basis for promiscuity and specificity during *Candida glabrata* invasion of host epithelia. *Proc. Natl. Acad. Sci. U.S.A.* 109: 16864–16869.
- Manzano-Lopez, J., Perez-Linero, A.M., Aguilera-Romero, A., Martin, M.E., Okano, T., Silva, D.V., Seeberger, P.H., Riezman, H., Funato, K., Goder, V., et al. (2015). COPII coat composition is actively regulated by luminal cargo maturation. *Curr. Biol.* 25: 152–162.
- Mösch, H.U. and Fink, G.R. (1997). Dissection of filamentous growth by transposon mutagenesis in *Saccharomyces cerevisiae*. *Genetics* 145: 671–684.
- Muszkiet, L., Fontaine, T., Beau, R., Mouyna, I., Vogt, M.S., Trow, J., Cormack, B.P., Essen, L.O., Jouvion, G., and Latge, J.P. (2019). The glycosylphosphatidylinositol-anchored DFG family is essential for the insertion of galactomannan into the β -(1,3)-glucan-chitin core of the cell wall of *Aspergillus fumigatus*. *mSphere* 4, <https://doi.org/10.1128/msphere.00397-19>.
- Oh, S.H., Smith, B., Miller, A.N., Staker, B., Fields, C., Hernandez, A., and Hoyer, L.L. (2019). Agglutinin-like sequence (ALS) Genes in the *Candida parapsilosis* species complex: blurring the boundaries between gene families that encode cell-wall proteins. *Front. Microbiol.* 10: 781.
- Oppler, Z.J., Parrish, M.E., and Murphy, H.A. (2019). Variation at an adhesin locus suggests sociality in natural populations of the yeast *Saccharomyces cerevisiae*. *Proc. Biol. Sci.* 286: 20191948.
- Orlean, P. (2012). Architecture and biosynthesis of the *Saccharomyces cerevisiae* cell wall. *Genetics* 192: 775–818.
- Peters, B.M., Ovchinnikova, E.S., Krom, B.P., Schlecht, L.M., Zhou, H., Hoyer, L.L., Busscher, H.J., van der Mei, H.C., Jabra-Rizk, M.A., and Shirtliff, M.E. (2012). *Staphylococcus aureus* adherence to *Candida albicans* hyphae is mediated by the hyphal adhesin Als3p. *Microbiology* 158: 2975–2986.
- Petosa, C., Collier, R.J., Klimpel, K.R., Leppla, S.H., and Liddington, R.C. (1997). Crystal structure of the anthrax toxin protective antigen. *Nature* 385: 833–838.
- Pittet, M. and Conzelmann, A. (2007). Biosynthesis and function of GPI proteins in the yeast *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* 1771: 405–420.
- Regenberg, B., Hanghoj, K.E., Andersen, K.S., and Boomsma, J.J. (2016). Clonal yeast biofilms can reap competitive advantages through cell differentiation without being obligatorily multicellular. *Proc. Biol. Sci.* 283, <https://doi.org/10.1098/rspb.2016.1303>.
- Reynolds, T.B. and Fink, G.R. (2001). Bakers' yeast, a model for fungal biofilm formation. *Science* 291: 878–881.
- Rigden, D.J., Mello, L.V., and Galperin, M.Y. (2004). The PA14 domain, a conserved all- β domain in bacterial toxins, enzymes, adhesins and signaling molecules. *Trends Biochem. Sci.* 29: 335–339.
- Rossouw, D., Bagheri, B., Setati, M.E., and Bauer, F.F. (2015). Co-flocculation of yeast species, a new mechanism to govern population dynamics in microbial ecosystems. *PLoS One* 10: e0136249.
- Salgado, P.S., Yan, R., Taylor, J.D., Burchell, L., Jones, R., Hoyer, L.L., Matthews, S.J., Simpson, P.J., and Cota, E. (2011). Structural basis for the broad specificity to host-cell ligands by the pathogenic fungus *Candida albicans*. *Proc. Natl. Acad. Sci. U.S.A.* 108: 15775–15779.
- Schimmoller, F., Singer-Kruger, B., Schroder, S., Kruger, U., Barlowe, C., and Riezman, H. (1995). The absence of Emp24p, a component of ER-derived COPII-coated vesicles, causes a defect in transport of selected proteins to the Golgi. *EMBO J.* 14: 1329–1339.
- Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., and Ideker, T. (2003). Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* 13: 2498–2504.
- Shihata, A.M.E.A. and Mrak, E.M. (1952). Intestinal yeast floras of successive populations of *Drosophila*. *Evolution* 6: 325–332.
- Silverman, R.J., Nobbs, A.H., Vickerman, M.M., Barbour, M.E., and Jenkinson, H.F. (2010). Interaction of *Candida albicans* cell wall Als3 protein with *Streptococcus gordonii* SspB adhesin promotes development of mixed-species communities. *Infect. Immun.* 78: 4644–4652.

- Sim, L., Groes, M., Olesen, K., and Henriksen, A. (2013). Structural and biochemical characterization of the N-terminal domain of flocculin Lg-Flo1p from *Saccharomyces pastorianus* reveals a unique specificity for phosphorylated mannose. *FEBS J.* 280: 1073–1083.
- Smukalla, S., Caldara, M., Pochet, N., Beauvais, A., Guadagnini, S., Yan, C., Vences, M.D., Jansen, A., Prevost, M.C., Latge, J.P., et al. (2008). *FLO1* is a variable green beard gene that drives biofilm-like cooperation in budding yeast. *Cell* 135: 726–737.
- Spohner, S.C., Schaum, V., Quitmann, H., and Czermak, P. (2016). *Kluyveromyces lactis*: an emerging tool in biotechnology. *J. Biotechnol.* 222: 104–116.
- Spreghini, E., Davis, D.A., Subaran, R., Kim, M., and Mitchell, A.P. (2003). Roles of *Candida albicans* Dfg5p and Dcw1p cell surface proteins in growth and hypha formation. *Eukaryot. Cell* 2: 746–755.
- Timmermans, B., De Las Penas, A., Castano, I., and Van Dijck, P. (2018). Adhesins in *Candida glabrata*. *J Fungi (Basel)* 4: 60.
- Umemura, M., Okamoto, M., Nakayama, K., Sagane, K., Tsukahara, K., Hata, K., and Jigami, Y. (2003). GWT1 gene is required for inositol acylation of glycosylphosphatidylinositol anchors in yeast. *J. Biol. Chem.* 278: 23639–23647.
- Vachova, L., Stovicek, V., Hlavacek, O., Chernyavskiy, O., Stepanek, L., Kubinova, L., and Palkova, Z. (2011). Flo11p, drug efflux pumps, and the extracellular matrix cooperate to form biofilm yeast colonies. *J. Cell Biol.* 194: 679–687.
- Vale-Silva, L.A., Moeckli, B., Torelli, R., Posteraro, B., Sanguinetti, M., and Sanglard, D. (2016). Upregulation of the adhesin gene EPA1 mediated by PDR1 in *Candida glabrata* leads to enhanced host colonization. *mSphere* 1, <https://doi.org/10.1128/msphere.00065-15>.
- Valotteau, C., Prystopiuk, V., Cormack, B.P., and Dufrene, Y.F. (2019). Atomic force microscopy demonstrates that *Candida glabrata* uses three epa proteins to mediate adhesion to abiotic surfaces. *mSphere* 4, <https://doi.org/10.1128/msphere.00277-19>.
- Van Mulders, S.E., Ghequire, M., Daenen, L., Verbelen, P.J., Verstreppe, K.J., and Delvaux, F.R. (2010). Flocculation gene variability in industrial brewer's yeast strains. *Appl. Microbiol. Biotechnol.* 88: 1321–1331.
- Vazquez, H.M., Vionnet, C., Roubaty, C., and Conzelmann, A. (2014). Cdc1 removes the ethanolamine phosphate of the first mannose of GPI anchors and thereby facilitates the integration of GPI proteins into the yeast cell wall. *Mol. Biol. Cell* 25: 3375–3388.
- Veelders, M., Brückner, S., Ott, D., Unverzagt, C., Mösch, H.U., and Essen, L.O. (2010). Structural basis of flocculin-mediated social behavior in yeast. *Proc. Natl. Acad. Sci. U.S.A.* 107: 22511–22516.
- Vogt, M.S., Schmitz, G.F., Silva-Varon, D., Mösch, H.U., and Essen, L.-O. (2020). Structural base for the transfer of GPI-anchored glycoproteins into fungal cell walls. *Proc. Natl. Acad. Sci. U.S.A.* 117: 22061–22067.
- Wang, C. and St Leger, R.J. (2007). The MAD1 adhesin of *Metarhizium anisopliae* links adhesion with blastospore production and virulence to insects, and the MAD2 adhesin enables attachment to plants. *Eukaryot. Cell* 6: 808–816.
- Willaert, R.G. (2018). Adhesins of yeasts: protein structure and interactions. *J Fungi (Basel)* 4, <https://doi.org/10.3390/jof4040119>.
- Xu, Z., Green, B., Benoit, N., Schatz, M., Wheelan, S., and Cormack, B. (2020). De novo genome assembly of *Candida glabrata* reveals cell wall protein complement and structure of dispersed tandem repeat arrays. *Mol. Microbiol.*, <https://doi.org/10.2210/pdb6tms/pdb>.
- Yoko, O.T., Umemura, M., Komatsuzaki, A., Ikeda, K., Ichikawa, D., Takase, K., Kanzawa, N., Saito, K., Kinoshita, T., Taguchi, R., et al. (2018). Lipid moiety of glycosylphosphatidylinositol-anchored proteins contributes to the determination of their final destination in yeast. *Gene Cell.* 23: 880–892.
- Zajac, D., Karkowska-Kuleta, J., Bochenska, O., Rapala-Kozik, M., and Kozik, A. (2016). Interaction of human fibronectin with *Candida glabrata* epithelial adhesin 6 (Epa6). *Acta Biochim. Pol.* 63: 417–426.
- Zallot, R., Oberg, N., and Gerlt, J.A. (2019). The EFI web resource for genomic enzymology tools: leveraging protein, genome, and metagenome databases to discover novel enzymes and metabolic pathways. *Biochemistry* 58: 4169–4182.
- Zara, S., Bakalinsky, A.T., Zara, G., Pirino, G., Demontis, M.A., and Budroni, M. (2005). *FLO11*-based model for air-liquid interfacial biofilm formation by *Saccharomyces cerevisiae*. *Appl. Environ. Microbiol.* 71: 2934–2939.
- Zhu, Y., Vionnet, C., and Conzelmann, A. (2006). Ethanolaminephosphate side chain added to glycosylphosphatidylinositol (GPI) anchor by mcd4p is required for ceramide remodeling and forward transport of GPI proteins from endoplasmic reticulum to Golgi. *J. Biol. Chem.* 281: 19830–19839.
- Zupancic, M.L., Frieman, M., Smith, D., Alvarez, R.A., Cummings, R.D., and Cormack, B.P. (2008). Glycan microarray analysis of *Candida glabrata* adhesin ligand specificity. *Mol. Microbiol.* 68: 547–559.