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Molecular phylogenetics of ascomycotal adhesins—A novel family of putative cell-surface adhesive proteins in fission yeasts

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Abstract

In this work, we identify a family of putative adhesins in the fission yeasts *Schizosaccharomyces pombe* and *Schizosaccharomyces japonicus*. The members of this family share a conserved tandem repeat related to those found in the *Candida albicans* Als family of adhesins. Unlike previously characterised adhesins that possess conserved ligand-binding domains at the N-terminus, this group of proteins carry ligand-binding domains at their C-termini. We demonstrate that one such domain—the uncharacterised GLEYA domain, is related to the lectin-like ligand-binding domain found in the *Saccharomyces cerevisiae* Flo proteins. Unlike the Flo and Als proteins, the fission yeast adhesins do not contain detectable glycosyl phosphatidyl inositol (GPI) membrane anchor signals to mediate their attachment to the cell wall, which may suggest a novel cell wall attachment mechanism. Further sequence analysis identified several putative adhesins in the sub-phylum of Pezizomycotina, where only a few adhesins have been described to date.

Keywords: Adhesin; Flocculation; Cell wall; *Schizosaccharomyces pombe*; Tandem repeat

1. Introduction

The ability to adhere to substrates and other cells is crucial to microbial organisms such as fungi. Cell-to-cell adhesion is required during mating when two cells of opposite mating type must be able to recognise and adhere to each other to allow conjugation and cellular fusion. The phenomenon of asexual aggregation into larger clumps of cells known as flocculation is observed when yeast cells are either starved for nutrients or are otherwise stressed. Flocculation is thought to be a survival strategy whereby the cells in the centre of the aggregation, or floc, will be better protected while awaiting better growth conditions (Verstrepen and Klis, 2006). The propensity for flocculation is a very significant property in brewing yeasts, where flocculating yeast cells can be conveniently separated from the fermentation product once all available sugars have been converted into ethanol. Pathogenic fungi often aggregate into specialised structures known as biofilms, which both enhance their ability to invade of host tissues and also serve to protect the pathogen from the immune system of the host. In addition fungal cells need to be able to adhere to their surroundings, be it either abiotic surfaces or living tissues, in order to remain firmly attached to their source of nutrients.

Adhesive properties in fungi are conveyed by a group of cell-surface proteins called adhesins (sometimes also referred to as agglutinins or flocculins, Dranginis et al., 2007). Several fungal adhesins have been described to date including the *Saccharomyces cerevisiae* Flo (flocculin) proteins (Teunissen and Steensma, 1995), the *Candida albicans* Als (agglutinin-like sequence) proteins (Hoyer, 2001), the *Candida glabrata* Epa (epithelial adhesion) proteins (Cormack et al., 1999; Kaur et al., 2005) and the *Pneumocystis carinii* major surface glycoproteins (Msg, Benfield and Lundgren, 1998). These proteins are generally rich in serine and threonine residues that allow extensive *O*-linked glycosylation. Some fungal adhesins also carry discernable ligand-binding domains that determine their substrate

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specificity and allows some degree of sequence classification of the different groups of adhesins (Frieman et al., 2002; Hoyer, 2001; Kobayashi et al., 1998; Sheppard et al., 2004). Pathogenic fungi are absolutely dependent on adhesins in order to recognise and adhere to host tissues (Cormack et al., 1999; Staab et al., 1999). Such adhesins can also mediate invasion of host tissues and even invasion of host cells (Sheppard et al., 2004). Understanding the biology of this group of proteins is therefore of great therapeutic interest.

Little is known about cell-surface adhesins in the fission yeast *Schizosaccharomyces pombe*. A number of regulators of asexual flocculation have been identified (Kim et al., 2001; Samuelsen et al., 2003; Tanaka et al., 1999; Tang et al., 2003; Watson and Davey, 1998). So far only two proteins with adhesive properties have been identified—the mating type-specific agglutinins Mam3 and Map4 (Mata and Bahler, 2006; Sharifmoghadam et al., 2006). *S. pombe* adhesins are thought to mainly contain galactosyl groups as asexual flocculation can be inhibited by the presence of galactose or lactose in the medium (Kim et al., 2001; Tanaka et al., 1999). It is known that ethanol-induced flocculation can be disrupted by protease treatment, which indicates that flocculation is mediated by proteins on the cell wall exterior (Geleta et al., 2007).

Adhesins as well as other cell wall proteins found within the Saccharomycotina predominantly require C-terminal glycosyl phosphatidyl inositol (GPI) membrane anchors for their subsequent attachment to the cell wall. Once the secreted protein reaches the membrane exterior, the GPI anchor is cleaved and the adhesin is then covalently linked to sugar moieties within the cell wall (Lu et al., 1994; Lu et al., 1995). Relatively few proteins in *S. pombe* are thought to be GPI-modified and neither Mam3 nor Map4 have detectable GPI anchor attachment signals (de Groot et al., 2003; Eisenhaber et al., 2004; Sharifmoghadam et al., 2006). A group of non-adhesive *S. cerevisiae* cell wall proteins known as Pir proteins (proteins with internal repeats) become attached to the cell wall in a GPI-independent manner. The five Pir proteins in *S. cerevisiae* (Pir1–Pir5) appear to play a role in cell wall integrity (Mrsa et al., 1997; Mrsa and Tanner, 1999) and are up-regulated during cell wall stress (Boorsma et al., 2004; Garcia et al., 2004). Studies have shown that Pir proteins become covalently linked to cell wall sugar molecules directly through glutamine residues within their tandem repeat domains (Castillo et al., 2003; Ecker et al., 2006). These links are sensitive to alkaline treatment, which allows them to be isolated separately from GPI-modified cell wall proteins (Mrsa et al., 1997). Although Pir proteins do not appear to be conserved in fission yeast, a recent proteomic study of the *S. pombe* cell wall found proteins that bound to the cell wall matrix through alkali-sensitive bonds in a manner similar to the *S. cerevisiae* Pir proteins (de Groot et al., 2007).

In this work, we identify a family of putative adhesins in *S. pombe* and the related fission yeast *Schizosaccharomyces japonicus*. The members of this family, which includes Mam3 and Map4, share a conserved tandem repeat with similarities to the tandem repeats found in the Als family of adhesins. The majority of the proteins in the Mam3/Map4 adhesin family carry conserved putative ligand-binding domains at their C-termini, some of which are also found in known adhesins. We show that one such domain—the GLEYA domain, is related to the lectin-like ligand-binding domain found in the *S. cerevisiae* Flo proteins and the *C. glabrata* Epa proteins. This family of adhesins does not appear to be dependent on C-terminal GPI anchors for their attachment to the cell wall. In addition, the C-terminal location of their ligand-binding domains would suggest an alternative form of cell wall attachment. We also identify several putative adhesins in the sub-phylum of Pezizomycotina, which encompasses the filamentous ascomycetes. Only a few adhesins have been described in the Pezizomycotina to date, none of which are found in other fungal lineages (Hogan et al., 1995; Hung et al., 2002; Newman et al., 1995; Wang and St Leger, 2007; Weaver et al., 1996).

2. Materials and methods

2.1. Sequence retrieval, alignment and analysis

All BLASTP and PSI-BLAST searches (Altschul et al., 1997) applied an expect value cut-off of 10^{-5} with the low-complexity region filter and composition-based adjustment of significance enabled unless specified otherwise. *Botrytis cinerea*, *Candida lusitaniae*, *Candida tropicalis*, *Fusarium graminearum* (anamorph *Gibberella zeae*), *Fusarium oxysporum*, *Fusarium verticillioides*, *Lodderomyces elongisporus* and *S. japonicus* sequences were retrieved from the Fungal Genome Initiative Database of the Broad Institute of Harvard and MIT (http://www.broad.mit.edu/annotation/fgi/). *Trichoderma reesei*, *Mycosphaerella graminicola* and *Fusarium solani* (anamorph *Nectria haematococca*) sequences were retrieved from the Joint Genome Institute (http://genome.jgi-psf.org/). All remaining sequences were collected from NCBI GenBank (http://www.ncbi.nlm.nih.gov/) with accession numbers listed in the relevant sections. Protein repeats were characterised using RADAR (Heger and Holm, 2000; http://www.ebi.ac.uk/Radar/). Protein sequences were aligned in MAFFT (Katoh et al., 2005) using the L-INS-i iterative refinement methodology (Ahola et al., 2006). Initial formatting of alignments was carried out in BOXSHADE (http://www.ch.embnet.org/software/BOX_form.html) followed by manual coloration. The following functional groupings were used: small hydrophobic residues (A, G) were shaded red; medium-sized hydrophobic residues (I, L, M, V) were shaded blue; hydroxyl group-containing residues (S, T) were shaded green; proline (P) was shaded yellow; cysteine (C) was shaded pink; aromatic residues (F, W, Y) were shaded violet; basic residues (H, R, K) were shaded turquoise; acidic residues (E, D) were shaded dark red; amide