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MINI-REVIEW

Diverse roles of Tup1p and Cyc8p transcription regulators in the development of distinct types of yeast populations

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Abstract

Yeasts create multicellular structures of varying complexity, such as more complex colonies and biofilms and less complex flocs, each of which develops via different mechanisms. Colony biofilms originate from one or more cells that, through growth and division, develop a complicated three-dimensional structure consisting of aerial parts, agar-embedded invasive parts and a central cavity, filled with extracellular matrix. In contrast, flocs arise relatively quickly by aggregation of planktonic cells growing in liquid cultures after they reach the appropriate growth phase and/or exhaust nutrients such as glucose. Creation of both types of structures is dependent on the presence of flocculins: Flo11p in the former case and Flo1p in the latter. We recently showed that formation of both types of structures by wild *Saccharomyces cerevisiae* strain BR-F is regulated via transcription regulators Tup1p and Cyc8p, but in a divergent manner. Biofilm formation is regulated by Cyc8p and Tup1p antagonistically: Cyc8p functions as a repressor of *FLO11* gene expression and biofilm formation, whereas Tup1p counteracts the Cyc8p repressor function and positively regulates biofilm formation and Flo11p expression. In addition, Tup1p stabilizes Flo11p probably by repressing a gene coding for a cell wall or extracellular protease that is involved in Flo11p degradation. In contrast, formation of BR-F flocs is co-repressed by the Cyc8p–Tup1p complex. These findings point to different mechanisms involved in yeast multicellularity.

Keywords Yeast multicellular structures · Colony biofilm · Flocculation · Cyc8p and Tup1p · Adhesion and invasion

Cyc8p and Tup1p in transition between colony and biofilm mode of multicellularity

Two major types of colony structures are formed by *Saccharomyces cerevisiae* strains: smooth colonies created by the majority of laboratory strains and structured colony biofilms formed by various wild strains isolated from natural environment (Palkova and Vachova 2016; Palkova et al. 2014; Vachova and Palkova 2018). Wild strains possess the ability to flip multicellular structure formation between smooth colony and structured biofilm modes, depending on nutrient availability. In addition, wild strains can undergo stable switching under rich nutritive conditions during which they turn off biofilm-specific traits and begin to form smooth colonies, independently of nutrient sources. This process, called domestication, is reversible (Stovicek et al. 2014). So far identified biofilm-specific traits that are switched off during domestication include cell adhesion to and invasion of semisolid material (such as agar), cell–cell adhesion via extracellular fibers, dependent on the presence of Flo11p adhesin and production of hydratable extracellular matrix (Stovicek et al. 2010, 2014; Vachova et al. 2011).

Recently, we discovered an important role of transcription regulators Cyc8p and Tup1p in regulation of biofilm-specific traits and thus in modulating colony complexity between smooth and biofilm modes (Nguyen et al. 2018). Cyc8p (Ssn6p) and Tup1p are conserved in eukaryotic organisms including mammals and have mainly been known as a transcriptional corepressor, consisting of four molecules of Tup1p and one molecule of Cyc8p (Smith and Johnson 2000; Varanasi et al. 1996). Later, Wong and Struhl (2011) proposed that Cyc8p–Tup1p repression occurs by masking

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activation domains and blocking activator-mediated recruitment of coactivators. Accordingly, Cyc8–Tup1p binding to DNA is mediated via its interaction with sequence-specific DNA-binding interacting proteins, both repressors and activators (Kliewe et al. 2017; Smith and Johnson 2000). By studying multicellular structures formed by *S. cerevisiae* strains with modified level of Cyc8p and Tup1p, we found that these transcription regulators antagonistically regulate the level of Flo11p and colony biofilm structure (Fig. 1a) (Nguyen et al. 2018). Overexpression of *CYC8* completely blocks *FLO11* expression and formation of organized extracellular fibers among cells, prevents cell adhesion to and penetration into agar and results in the formation of smooth colonies. Unexpectedly, the same cell behavior was identified when the *TUP1* gene was deleted, implying opposing effects of Cyc8p and Tup1p on biofilm-specific traits and the development of complex biofilms. Further, in-depth analyses of changes at different *FLO11* expression levels (mRNA and protein) in strains in which the *TUP1* and *CYC8* genes were controlled by inducible promoters, revealed that Cyc8p is the major repressor of *FLO11*, while Tup1p counteracts Cyc8p function and thus induces, probably indirectly, *FLO11* expression (Nguyen et al. 2018). As Tup1p and Cyc8p are able to form a co-repressor complex that apparently has a different function (see below), relative levels of Cyc8p and Tup1p can fine-tune the level of free Cyc8p, needed for *FLO11* gene repression. The level of Cyc8p repressor could also be influenced by formation of Cyc8p-prion, an inactive form of Cyc8p, as hypothesized by Patel et al. (2009). However, counteracting the Cyc8p repressor function is not the only role of Tup1p in colony biofilm formation. Tup1p is also essential for regulating levels of Flo11p molecules by protecting them against degradation, possibly via Tup1p-dependent repression of a cell wall or extracellular protease that degrades Flo11p (Fig. 1a) (Nguyen et al. 2018). Such a protease is yet to be identified but we can speculate that it may be involved in Flo11p shedding and its subsequent degradation and, thus in balancing mutual levels of cell wall attached and free/released Flo11p within the colony/biofilm. Such a protease could also degrade Flo11p in those parts of colonies where its presence is not required. Further studies of the dynamics of Flo11p accumulation within the colony structure are needed to clarify this point. Differences in Flo11p processing were found in a strain defective in the serine protease Kex2p (Karunanithi et al. 2010), but Flo11p does not contain prominent Kex2p cleavage sites (Bader et al. 2008) and, therefore, is probably not a direct target of Kex2p. So, potentially, a protease that cleaves Flo11p could itself be processed via Kex2p. In addition, studies of Tup1p orthologues showed that Tup1p represses production of secreted aspartyl proteinases (SAPs) in *Candida albicans* (Naglik et al. 2004) and extracellular proteases in *Aspergillus nidulans* (Schachtschabel et al. 2013).

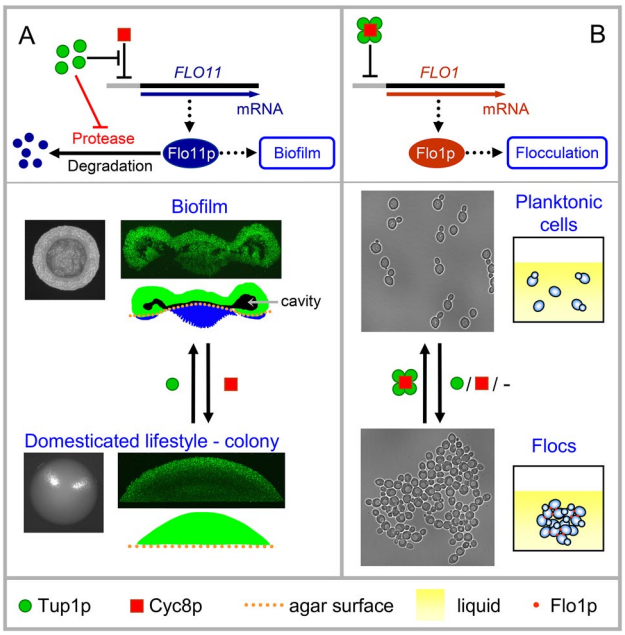

Fig. 1 Scheme of Tup1p and Cyc8p regulation of multicellular processes in *S. cerevisiae*. **a** Antagonistic regulation by Tup1p and Cyc8p of biofilm and domesticated (colony) lifestyles. Cyc8p represses expression of *FLO11* gene encoding the major player in biofilm formation; Tup1p positively regulates Flo11p formation by antagonizing Cyc8p-mediated repression and by stabilization of Flo11p by repressing a protease involved in Flo11p degradation. Aerial views and side views obtained by two-photon excitation confocal microscopy of biofilm/colony cross sections stained with Calcofluor white (false green color) are shown, together with respective schemes. Adapted from Nguyen et al. (2018). **b** Synergistic regulation by the Tup1p–Cyc8p co-repressor of multicellular floc formation. Co-repressor complex of four Tup1p and one Cyc8p molecules represses formation of flocculin Flo1p. Absence of either Tup1p or Cyc8p or both results in the formation of Flo1p, the presence of which causes cell flocculation and formation of multicellular aggregates (Fleming et al. 2014; Nguyen et al. 2018). Bright field microscopic images of planktonic cells and cells forming flocs are adapted from Nguyen et al. (2018)

Cyc8p and Tup1p in yeast adhesion and invasion in *S. cerevisiae* and yeast/fungal pathogens

Cell adhesion to and invasion into a semisolid support are important processes in the formation of multicellular structures and are also related to virulence of pathogenic yeast/fungi. The involvement of Tup1p and Cyc8p proteins in these processes has been demonstrated in various yeast/fungi, but the findings are rather inconsistent and imply differences in Tup1p/Cyc8p functions in particular species (or even strains), growth conditions and/or stages of filamentous cell formation.

In *S. cerevisiae*, similar to inhibition of biofilm colony formation, cell adhesion and pseudohyphae invasion of the wild strain are also completely blocked by Cyc8p overexpression and *TUP1* deletion and thus antagonistically regulated by Cyc8p and Tup1p (Nguyen et al. 2018). On the other hand, deletion of *TUP1* stimulated adhesion to plastic and to agar and increased *FLO1* and *FLO11* mRNA levels in laboratory strain BY4741 (S288c background), which is impaired in haploid adhesion, biofilm formation and diploid pseudohyphal growth, partially due to non-functional *FLO8* (Fichtner et al. 2007).

In *C. albicans*, filamentous growth and hypha-specific genes were repressed by Tup1p. Accordingly, deletion of *TUP1* increased *C. albicans* filamentation and surface invasion and the strains formed wrinkled invasive colonies (Braun and Johnson 1997; Garcia-Sanchez et al. 2005, Su et al. 2018). Ssn6p (a Cyc8p orthologue) was also reported to be a repressor of filamentation and of wrinkled colony morphology under particular conditions and some of these repressive effects were enhanced by deletion of the gene, coding for histone deacetylase Rpd31p (Lee et al. 2015). However, other reports implicate Ssn6p in different types of phenotypic switching rather than in filamentous growth. *ssn6* strains displayed an unstable phenotype and switched between different forms, with partial changes in cell and colony morphology. It was, therefore, concluded that Ssn6p may act as a repressor of phenotypic switching (Garcia-Sanchez et al. 2005). In addition, roles were identified for both Ssn6p and Tup1p in white-opaque switching of *C. albicans*: Ssn6p being a negative regulator of the opaque transcription program in white cells and of the white transcription program in opaque cells (Hernday et al. 2016) and Tup1p as a repressor of the opaque state, which, together with its negative regulator Wor1p, controls the opaque switch under different circumstances (Alkafeef et al. 2018). In summary, both Tup1p and Ssn6p seem to contribute to repression of *C. albicans* filamentation, at least partially independently of one another and dependently on growth conditions and dependently on the functions of other interacting regulators. Hence, Kim et al. (2015) hypothesize that the interplay between Ssn6p and Tup1p, either in a complex or as individual proteins, and other regulatory proteins (e.g., Nrg1p or Rpd3p) differently regulates hyphal-specific genes under different stages of the dimorphic transition from yeast to hyphal form (yeast state, hyphal induction and hyphal maintenance and extension).

As to other yeast/fungi, Tup1p is indispensable for yeast to hypha transition in the maize pathogen *Ustilago maydis*; accordingly *TUP1* deletion reduces filamentation and virulence of this fungus (Elias-Villalobos et al. 2011). Similarly, in *Penicillium marneffei*, TupA is required for cell filamentation, and in *Cryptococcus neoformans* for the formation of dikaryotic hyphae and virulence (Lee et al. 2005, 2009; Todd et al. 2003). Mo*TUP1* was recently identified in *Magnaporthe oryzae* (a rice pathogen), and its deletion caused decreased production of aerial hyphae and decreased pathogenicity of the fungus (Chen et al. 2015; Li et al. 2017). Hence, contrary to *C. albicans* and in agreement with *S. cerevisiae* wild strains, Tup1p seems to function as an activator of filamentation in these yeast/fungi.

Cyc8p and Tup1p in yeast flocculation

Cell flocs, mostly studied in the yeast *S. cerevisiae*, represent a different type of yeast multicellular population (Smukalla et al. 2008). Flocs are formed by flocculation of planktonic cells within liquid yeast cultures depending not only on the presence of flocculin Flo1p (the major flocculin), but also on other Ca^{2+}-dependent lectin-type flocculins such as Flo10p (Verstrepen and Klis 2006). Cell flocculation of flocculent strains is dependent on nutrient availability as it can be induced by nutrient limitation of mainly sugars and nitrogen sources (Soares 2011; Teunissen et al. 1995). It is, therefore, usually initiated at the end of exponential or during stationary phase. Formation of multicellular flocs is regulated by Tup1p and Cyc8p differently from regulation of biofilm formation (Fig. 1) (Lipke and Hullpillsbury 1984; Nguyen et al. 2018; Stratford 1992; Teunissen et al. 1995). Impairment of any of these regulators induces floc formation even under non-flocculation conditions and increases, by ~ five times, the flocculation of wild strains (Fig. 1b) (Nguyen et al. 2018). A model was proposed, in which the Tup1p–Cyc8p co-repressor complex binds to a distinct site at the *FLO1* promoter (Chen et al. 2017; Fleming et al. 2014). This subsequently brings to the promoter region the histone deacetylases Hda1p and Rpd3p, which deacetylate histones in nucleosomes over the *FLO1* promoter and upstream regions and thus repress *FLO1* expression. In the absence of Cyc8p–Tup1p, chromatin structure over the *FLO1* promoter is remodeled, leading to *FLO1* de-repression and subsequent cell flocculation. Regulation of flocculation in other yeast is less well studied; speculations exist that flocculation of *C. albicans* cells is repressed by Sfl1p in concert with Tup1p and Nrg1p (Bauer and Wendland 2007).

In summary, Cyc8p and Tup1p play important pleiotropic roles in yeast multicellularity, being important regulators of key processes, contributing to different aspects of/stages in the formation of various structured populations (Nguyen et al. 2018). Differences in Cyc8p/Tup1p functions observed between different species/strains may reflect either evolutionary functional divergence of these regulators, or, more probably, the fact that the multicellular structures are complex and plastic and processes contributing to their formation, such as filamentation, are not uniform. For example, various types of filaments can be distinguished

among the species and nutritive conditions, which can play different roles in different processes, such as cell invasion involved in attachment of a structure to a substrate or cell extension from a structure, involved in occupation of new niches (Vopalenska et al. 2010). Moreover, different stages of these processes have to be controlled. Cyc8p and Tup1p have been described as interacting and forming complexes with numerous additional regulators and chromatin remodeling proteins, each of which could modify the functions of Cyc8p and/or Tup1p proteins in respect to a particular process under distinct circumstances. In this regard, more information on distinct Cyc8p and/or Tup1p complexes and, in particular, on their functional dynamics during structured population development is required to resolve the complex regulatory networks among these proteins and their roles in yeast multicellularity.

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