ABSTRACT

Biofilms are a common mode of yeast growth in which cells adhere to each other and adhere to biotic and abiotic surfaces to form complex multicellular structures. Living together in biofilms provides cells with several benefits, compared to planktonic cells such as protection and resistance to antimicrobials, environmental stresses and host immune attacks. Biofilms may play many important roles in commercial industries. But they are considered to be extremely dangerous in clinical settings. There is thus great interest in studying biofilms and how to eliminate them.

In this study, we used wild yeast *Saccharomyces cerevisiae* colony biofilm as an ideal system to investigate potential functions of the yeast Cyc8p-Tup1p transcriptional corepressor complex in the regulation of yeast adhesion and biofilm formation on agar and at solid-liquid interfaces. Unexpectedly, we found that Cyc8p and Tup1p antagonistically control *FLO11* expression and the formation of structured biofilm colonies on agar. Cyc8p itself acts as a key repressor of *FLO11* and biofilm colony formation, whereas Tup1p promotes the formation of biofilm colonies and induces *FLO11* expression by inhibiting the repressive function of Cyc8p and preventing Flo11p degradation possibly by inhibiting an extracellular protease. Other typical features of biofilm colonies such as formation of fibers inter-connecting the cells and cell invasiveness, are inversely regulated by Cyc8p and Tup1p as well. On the other hand, both proteins in concert repress cell flocculation as reduced expression of either *CYC8* or *TUP1* led to the production of macroscopic flocs (clusters of cells).

The antagonistic actions of Cyc8p and Tup1p were also exhibited in the formation of solid-liquid interface biofilms. We have provided experimental evidence that Cyc8p and Tup1p are key regulators in two steps of the *S. cerevisiae*

biofilm developmental life cycle-cell adhesion (followed by biofilm formation) and biofilm dispersal. The first step, adhesion and biofilm organization, is conversely regulated by Tup1p (activator) and Cyc8p (repressor), whereas biofilm dispersal is controlled by Cyc8p and is dependent on the level of environmental glucose. We show that even a low level of glucose is sufficient to disrupt the biofilm and release planktonic cells.

Our proteomic data not only identified hundreds of genes known to be regulated by Cyc8p and Tup1p, but identified for the first time several extra sets of genes encoding proteins that are involved in processes such as protein refolding and protein complex assembly, chronological cell ageing and apoptosis. The data indicated that global effects of Cyc8p and Tup1p on the regulation of gene expression in yeast. Where Cyc8p and Tup1p may act together or act independently to control gene expression. Even more interesting, they can act oppositely in regulation of several target genes such as *FLO11, MET17* and *URA2.* Findings in this study confirmed that Flo11p is a key factor in abiotic adhesion and biofilm formation and other typical features of biofilm colonies that are positively regulated by Tup1p and negatively controlled by Cyc8p.