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Regulation of mating in the budding yeast *Saccharomyces cerevisiae* by the zinc cluster proteins Sut1 and Sut2 ☆

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ABSTRACT

The zinc cluster proteins Sut1 and Sut2 play a role in sterol uptake and filamentous growth in the budding yeast *Saccharomyces cerevisiae*. In this study, we show that they are also involved in mating. Cells that lack both *SUT1* and *SUT2* were defective in mating. The expression of the genes *NCE102* and *PRR2* was increased in the *sut1 sut2* double deletion mutant suggesting that Sut1 and Sut2 both repress the expression of *NCE102* and *PRR2*. Consistent with these data, overexpression of either *SUT1* or *SUT2* led to lower expression of *NCE102* and *PRR2*. Furthermore, expression levels of *NCE102*, *PRR2* and *RHO5*, another target gene of Sut1 and Sut2, decreased in response to pheromone. Prr2 has been identified as a mating inhibitor before. Here we show that overexpression of *NCE102* and *RHO5* also reduced mating. Our results suggest that Sut1 and Sut2 positively regulate mating by repressing the expression of the mating inhibitors *NCE102*, *PRR2* and *RHO5* in response to pheromone.

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1. Introduction

Sut1 and Sut2 of the budding yeast *Saccharomyces cerevisiae* are transcriptional regulators of the $Zn(II)_2Cys_6$ family, which is also known as zinc cluster family [1,2]. The two corresponding genes are paralogs that were formed by whole genome duplication [3]. Both proteins are involved in sterol import and filamentous growth but molecular mechanisms have only been characterized for Sut1 [2,4–6]. Sut1 represses the expression of genes such as *DAN1* and *AUS1*, which mediate sterol import, in the presence of oxygen and triggers their expression under anaerobic conditions [7–9]. The Cdc42 effector Ste20 binds to Sut1 and regulates sterol uptake via Sut1 [10].

Overexpression of either *SUT1* or *SUT2* leads to inhibition of filamentous growth suggesting that the corresponding proteins are negative regulators of filamentation [5,6]. Sut1 binds to the promoter regions of *GAT2*, *HAP4*, *MGA1*, *MSN4*, *NCE102*, *PRR2*, *RHO3*, and *RHO5*, and increased *SUT1* levels result in lower expression of these genes [6,11]. With the exception of *MGA1*, all genes are upregulated during filamentous growth [6]. Furthermore, *GAT2*, *HAP4*, *MGA1*, *RHO3* and *RHO5* are essential for filamentation [6,12,13]. Taken together, this suggests that Sut1 partially represses the expression of these genes in vegetatively growing cells. Under conditions that favor filamentous growth, this repression is relieved and the increased expression of the Sut1 target genes contributes to filamentation.

Here, we show that Sut1 and Sut2 also regulate mating. Haploid cells of opposite mating types have the ability to fuse and become diploid [14]. Both haploid mating types secrete a pheromone that can bind to a G-protein coupled receptor of the opposite cell type. This triggers a mitogen-activated protein cascade that results in a change of the gene expression pattern. Cells grow towards each other by forming a mating projection which allows them to fuse. In this study, we investigated the role of Sut1 and Sut2, and their target genes in mating.

2. Materials and methods

2.1. Yeast strains, plasmids and growth conditions

All yeast strains used in this study are listed in Supplementary Table S1. Yeast strains were grown in 1% yeast extract, 2% peptone and 2% dextrose or synthetic complete medium. Yeast strains were constructed using PCR-amplified cassettes [15]. A *LEU2*-marked *FUS1-lacZ* reporter was integrated at the *FUS1* locus using SphI-digested pFC23 [16], which was kindly provided by Peter Pryciak. All constructs used in this work are listed in Supplementary Table S2.

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2.2. Quantitative mating assays

3×10^6 exponentially growing cells of each mating type were mixed and collected on nitrocellulose filters. The filters were placed on yeast extract, peptone and dextrose plates for 4 h at 30 °C. Filters were then suspended in water and serial dilutions were plated on selective medium plates to determine the number of diploids. Mating efficiency was calculated as the percentage of input cells that formed diploids.

2.3. β-Galactosidase assays

Densities of exponentially growing cell cultures were measured by optical density at 600 nm (A_{600}). 0.1–10 ml of cells were harvested by centrifugation and resuspended in 1 ml Z buffer (100 mM sodium phosphate pH 7.0, 10 mM KCl, 1 mM $MgSO_4$, 50 mM β-mercaptoethanol). Cells were permeabilized by addition of 20 μl chloroform and 20 μl 0.1% SDS. After 15 min incubation at 30 °C the reaction was started by addition of 140 μl *o*-nitrophenyl-β-D-galactopyranoside (4 mg/ml in 100 mM sodium phosphate, pH 7.0), incubated at 30 °C until the solution became yellow and the reaction was stopped by addition of 400 μl 1 M Na_2CO_3. Samples were centrifuged and absorbance of the supernatant at 420 nm and 550 nm was determined. β-galactosidase activity was calculated in Miller units as $1000 \times [A_{420} - (1.75 \times A_{550})]/[\text{reaction time (min)} \times \text{culture volume (ml)} \times A_{600}]$. To analyse gene expression in response to pheromone, cells were incubated with 1 μg/ml α-factor for 150 min.

3. Results

Sut1 was identified as a protein that physically interacts with the Cdc42 effector Ste20 [10]. Since Ste20 is essential for mating [17,18] we wanted to know whether Sut1 has a role in this processes as well. The deletion of *SUT1* had no effect on mating efficiency (Fig. 1A). Likewise, cells lacking the paralog *SUT2* are indistinguishable from the wild type (Fig. 1A). In contrast, mating efficiency was considerably reduced for the *sut1*Δ *sut2*Δ double mutant (Fig. 1A), suggesting that both corresponding proteins play a positive role in mating. Transcriptional induction in response to pheromone was examined using *FUS1* as a marker. *FUS1* expression was determined by fusing the *FUS1* promoter to the *lacZ* gene. No effect was observed for the single mutants whereas *FUS1* expression was reduced in cells lacking both *SUT1* and *SUT2* (Fig. 1B). The formation of mating projections was also examined but this process was not affected by the deletion of *SUT1* and *SUT2* (data not shown).

Sut1 controls the expression of several genes [6,7,9]. We tested whether any of them change their expression in response to pheromone treatment. Sut1 induces the expression of *DAN1* and *AUS1*, whose products mediate sterol uptake from the extracellular medium, in the absence of oxygen [7–9]. *DAN1* and *AUS1* expression is not detectable in vegetatively growing cells under aerobic conditions [8,19]. Upon α-factor treatment, these genes were also not expressed (data not shown). The role of Sut1 in mating therefore seems to be independent of sterol import. Sut1 binds to the promoters of *GAT2*, *HAP4*, *MGA1*, *MSN4*, *NCE102*, *PRR2*, *RHO3* and *RHO5*, and the expression levels of all of these genes are decreased in cells overexpressing *SUT1* [6,11]. The expression of *NCE102*, *PRR2* and *RHO5* is reduced twofold in response to pheromone (Fig. 2) whereas this treatment has no effect on the levels of the other

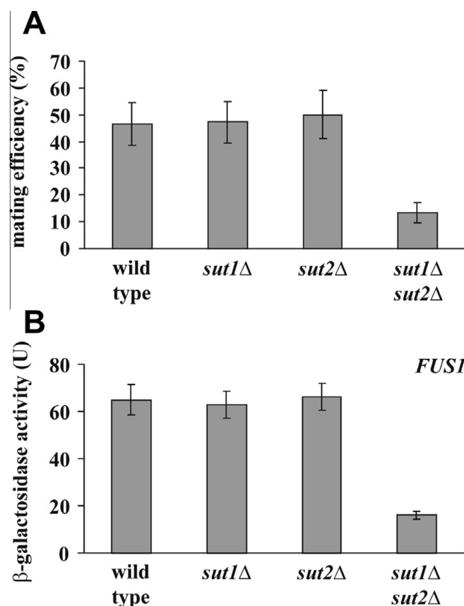

Fig. 1. Sut1 and Sut2 play a role in mating. (A) Deletion of *SUT1* and *SUT2* results in a reduced mating efficiency. The mating efficiency is given as the mean of three independent experiments with standard deviation bars. (B) *FUS1* expression is decreased in the *sut1*Δ *sut2*Δ double mutant. Shown is the mean of β-galactosidase activity with standard deviation ($n \geqslant 4$).

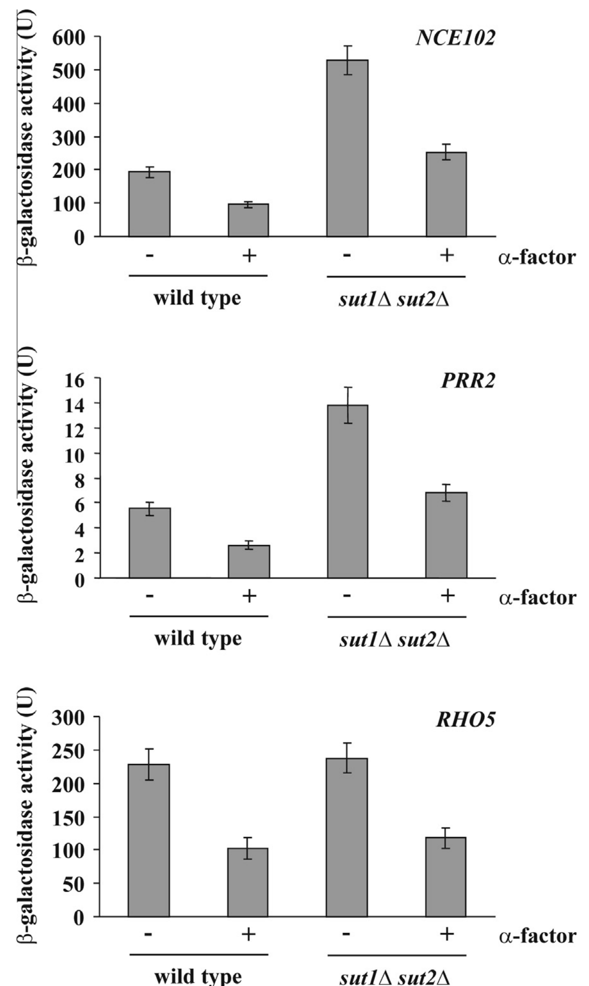

Fig. 2. Expression of Sut1 target genes in response to pheromone. Expression of *NCE102*, *PRR2* and *RHO5* with and without α-factor in the indicated strains was quantified using β-galactosidase assays. Shown is the mean β-galactosidase activity with standard deviation of at least four independent experiments.

Sut1 target genes (data not shown). The pheromone-induced change in expression for *NCE102*, *PRR2* and *RHO5* suggests that these genes might have a role in mating. We therefore focused on these genes for this study.

Since we established that Sut1 and Sut2 are both involved in mating, we next tested whether Sut2 also regulates the expression of *NCE102*, *PRR2* and *RHO5*. Cells overexpressing *SUT2* under control of the strong *PMA1* promoter from a multicopy plasmid displayed a 2- to 3-fold reduction of expression levels for *NCE102*, *PRR2* and *RHO5* (Fig. 3A). This suggests that Sut2 like Sut1 negatively regulates the expression of these genes. However, using the same type of overexpression, increased *SUT1* levels had a more pronounced effect on the expression of these genes (5.1-fold reduction for *NCE102*, 8.9-fold for *PRR2* and 3.9-fold for *RHO5*) [6]. The observed effect is gene-specific as increased *SUT2* levels do not affect the expression of the control gene *RHO4* (Fig. 3A). It was also tested whether deletion of *SUT1* and *SUT2* has an effect on the expression of *NCE102*, *PRR2* and *RHO5*. An increased expression of *NCE102* and *PRR2* but not of *RHO5* was observed for the *sut1*Δ *sut2*Δ strain (Fig. 3B). Deletion of *SUT1* had no effect on the expression of *NCE102* whereas *SUT2* deletion resulted in higher *NCE102* levels (Fig. 3B). Notably, *NCE102* expression in the *sut1*Δ *sut2*Δ double mutant is slightly but significantly ($P < 0.01$) higher compared to the *sut2*Δ strain (Fig. 3B). For *PRR2*, expression in the single deletion strains was comparable to the wild type (Fig. 3B). Together these data suggest that Sut1 and Sut2 both negatively regulate the expression of *NCE102* and *PRR2*.

Next, the pheromone-induced expression change of *NCE102*, *PRR2* and *RHO5* observed for wild type cells was compared with the expression of these genes in the mating-defective *sut1*Δ *sut2*Δ

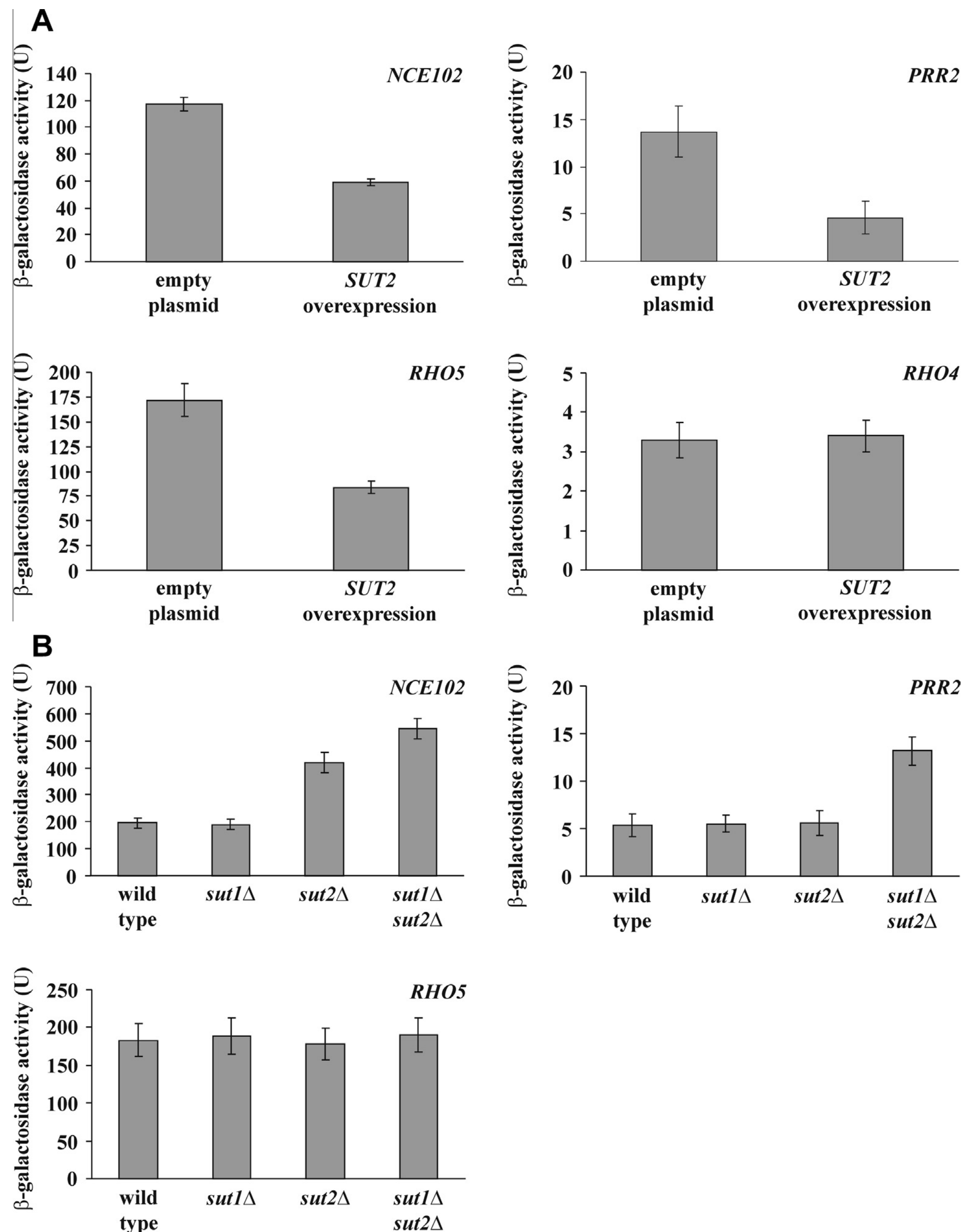

Fig. 3. Regulation of *NCE102*, *PRR2* and *RHO5* expression by Sut1 and Sut2. (A) *SUT2* overexpression results in decreased expression levels of *NCE102*, *PRR2* and *RHO5*. Cells harbored either a *SUT2* overexpression construct (pMC10) or an empty plasmid (pNEV-N) in combination with the *lacZ* reporter fused to the indicated promoter regions. Shown is the mean β-galactosidase activity with standard deviation ($n \geqslant 4$). (B) Expression of *NCE102*, *PRR2* and *RHO5* in the *sut1*Δ *sut2*Δ double mutant. The indicated strains carried *lacZ* reporter fused to the indicated promoter regions. Shown is the mean with standard deviation for at least four independent experiments.

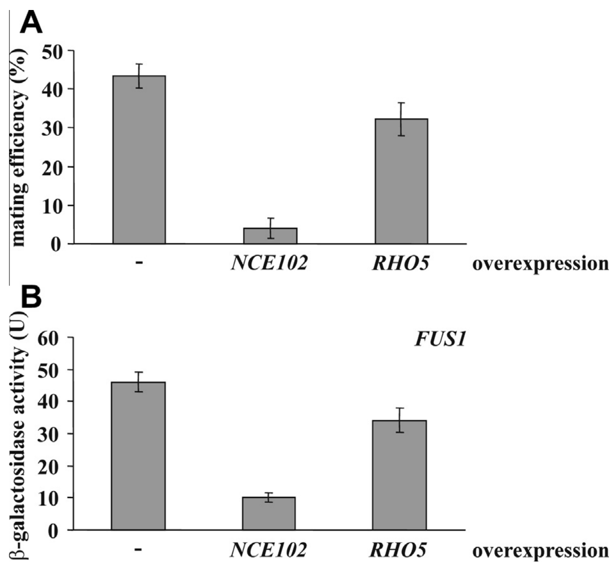

Fig. 4. Phenotype of cells overexpressing *NCE102* and *RHO5*. (A) Increased *NCE102* and RHO5 levels result in reduced mating efficiency. β-galactosidase activity was determined for cells overexpressing either *NCE102* or *RHO5* from multi-copy plasmids (pTH400 and pTH401) and for cells harboring the empty plasmid (pRS426). Shown is the mean β-galactosidase activity with standard deviation ($n \geqslant 4$). (B) *FUS1* expression of cells overexpressing *NCE102* and *RHO5*. Shown is the mean β-galactosidase activity with standard deviation of at least four independent experiments.

strain. As in the wild type, the expression of all three genes in cells lacking *SUT1* and *SUT2* was reduced twofold upon incubation with α-factor (Fig. 2). However, since the expression levels of *NCE102* and *PRR2* in the *sut1Δ sut2Δ* double mutant were higher compared to the wild type, the expression of these genes in *sut1Δ sut2Δ* cells treated with pheromone are similar to expression levels in untreated wild type cells (Fig. 2). The fact that *NCE102*, *PRR2* and *RHO5* levels decrease in response to pheromone treatment suggests that these factors function as mating inhibitors. It might be necessary to downregulate the expression of these genes for efficient mating. The high expression of genes such as *NCE102* and *PRR2* in *sut1Δ sut2Δ* cells would explain the mating defect of this double mutant. In fact, *PRR2* overexpression has previously been shown to inhibit mating and *FUS1* expression [20]. We tested whether *NCE102* and *RHO5* also act as mating inhibitors by cloning these genes under the control of their own promoters in multicopy plasmids. *RHO5* overexpression resulted in a slight but significant ($P < 0.05$) reduction of mating efficiency and *FUS1* expression (Fig. 4A and B). In contrast, mating efficiency and *FUS1* induction was considerably reduced in cells with increased *NCE102* levels (Fig. 4A and B). Thus, Nce102 and to a lesser extent Rho5 act as mating inhibitors like Prr2 and a reduced expression of the corresponding genes seems to be a prerequisite for mating.

4. Discussion

It has previously been shown that the transcriptional regulators Sut1 and Sut2 play a role in sterol import and filamentation [2,4–6]. Here, we demonstrate that Sut1 and Sut2 also regulate mating. In contrast to Sut1, very little is known about Sut2 and target genes have not been identified for Sut2. Our *SUT2* overexpression data indicate that Sut1 and Sut2 are not only involved in the same biological processes but that Sut2 also represses the expression of the Sut1 targets *NCE102*, *PRR2* and *RHO5*. Consistently, *NCE102* and *PRR2* are upregulated in cells lacking both *SUT1* and *SUT2*. Such an effect was not observed for *RHO5* expression. This can easily be explained by regulation by multiple transcription factors. Sut1 and Rho5 both have important roles in filamentous growth. Not only Sut1 but at least 6 other transcription factors involved in filamentation bind to the *RHO5* promoter [11,21]. It is therefore not surprising that due to this complex regulation the deletion of one or two transcription factors has no effect on *RHO5* expression.

The downregulation of *NCE102*, *PRR2* and *RHO5* upon mating pheromone treatment suggests that the corresponding proteins function as mating inhibitors. Such a role has already been described for Prr2, a serine/threonine-specific kinase that interferes with pheromone signalling by an unknown mechanism [20]. Here, we show that Nce102 and to a lesser extent Rho5 negatively regulating mating. Nce102 is involved in the formation of eisosomes, protein complexes that mediate the organization of the plasma membrane into specialized domains [22]. It would be interesting to examine whether eisosomes have a role in pheromone signalling at the plasma membrane. Rho5 is a component of several signalling pathways including the cell integrity which plays a crucial role in cell wall stability during formation of mating projections [23,24].

We propose that the downregulation of mating inhibitors such as Nce102, Prr2 and Rho5 is necessary for efficient mating. If expression levels remain high, for example in cells overexpressing these genes or in the *sut1Δ sut2Δ* double mutant, mating is reduced. In budding cells the expression of *NCE102*, *PRR2* and *RHO5* is only partially repressed. Pheromone signalling probably increases this repression mediated by Sut1 and Sut2 by an unknown mechanism. Interestingly, Sut1 and its targets have opposite effects during filamentation [6]. Sut1 is a negative regulator of filamentous growth, and *NCE102*, *PRR2* and *RHO5* are all upregulated during filamentous growth. Furthermore, *RHO5* is essential for filamentation. Deletion of either *NCE102* or *PRR2* has no effect on filamentation, possibly because both genes have paralogs [3]. In summary, it seems that Sut1 regulates several differentiation pathways in budding yeast. For filamentation Sut1-mediated repression is relieved whereas for mating this repression increases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.07.027.

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