Ecm22 and Upc2 regulate yeast mating through control of expression of the mating genes PRM1 and PRM4

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ABSTRACT

Budding yeast mating is an excellent model for receptor-activated cell differentiation. Here we identify the related transcription factors Ecm22 and Upc2 as novel regulators of mating. Cells lacking both ECM22 and UPC2 display strong mating defects whereas deletion of either gene has no effect. Ecm22 and Upc2 positively regulate basal expression of PRM1 and PRM4. These genes are strongly induced in response to mating pheromone, which is also largely dependent on ECM22 and UPC2. We further show that deletion of PRM4 like PRM1 results in markedly reduced mating efficiency. Expression of PRM1 but not of PRM4 is also regulated by Ste12, a key transcription factor for mating. STE12 deletion lowers basal PRM1 expression, whereas STE12 overexpression strongly increases PRM1 levels. This regulation of PRM1 transcription is mediated through three Ste12-binding sites in the PRM1 promoter. Simultaneous deletion of ECM22 and UPC2 as well as mutation of the three Ste12-binding sites in the PRM1 promoter completely abolishes basal and pheromone-induced PRM1 expression, indicating that Ste12 and Ecm22/Upc2 control PRM1 transcription through distinct pathways. In summary, we propose a novel mechanism for budding yeast mating. We suggest that Ecm22 and Upc2 regulate mating through the induction of the mating genes PRM1 and PRM4.

Keywords: Budding yeast, Mating, Zinc cluster protein, Transcription factor Ecm22, Upc2

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1. Introduction

The mating response of the unicellular budding yeast Saccharomyces cerevisiae is an ideal model for the study of receptor-activated cell differentiation [1–3]. Two different haploid cell types of budding yeast exist, termed a cells and α cells. When two cells of these opposite mating are in close proximity they can fuse to become diploid. The two haploid cell types secrete different peptide pheromones (α-factor and α-factor) which bind to a G protein-coupled receptor in the plasma membrane of the opposite cell type. This triggers the activation of a mitogen-activated protein kinase (MAPK) pathway which ultimately results in the induction of mating-specific genes, cell cycle arrest, polarized growth towards the mating partner and cell fusion. The transcription factor Ste12 plays a key role in the increased expression of mating-specific genes in response to pheromone [4–6].

In this study, we show that the transcription factors Ecm22 and Upc2 are novel regulators of mating. Ecm22 and the closely related Upc2 are both members of the zinc cluster protein family [7–10]. Initially, these transcription factors have been identified as key regulators of sterol import [8,9,11] and sterol biosynthesis [12,13]. Later it has been shown that Ecm22 and Upc2 also control filamentous growth [14], a response to nutrient limitation [15].

Ste12 and its paralog Sut2 are also transcription factors of the zinc cluster protein family [7,10,16]. Even though Ste12 and Sut2 are not related to Ecm22 and Upc2 they also control sterol uptake and filamentation [16–19]. Interestingly, Ecm22/Upc2 and Sut1/Sut2 regulate sterol import by induction of a very similar set of genes [13,20–24], and they share some target genes for the regulation of filamentation [14,19].

We have previously shown that Sut1/Sut2 also regulate mating [25]. Here, we show that Ecm22 and Upc2 control mating through the regulation of expression of the genes PRM1 and PRM4 which are strongly upregulated during mating and which are required for efficient mating.

2. Materials and methods

2.1. Yeast strains and growth conditions

All yeast strains used in this study are listed in Table 1.
Simultaneous deletion of ECM22 and UPC2 is lethal in some yeast strains [9]. In this study, we used the Σ1278b background because here the lack of ECM22 and UPC2 has no effect on the growth rate [14]. Yeast strains were constructed using PCR-amplified cassettes [26–28]. Yeast strains were grown in 1% yeast extract, 2% peptone, 2% dextrose (YPD) or synthetic complete (SC) medium. For STE12 overexpression, yeast cells were grown in medium with 2% galactose and 3% raffinose instead of glucose. To analyse gene expression in response to pheromone, cells were incubated with 10 μg/ml α-factor for 150 min.

2.2. Generation of plasmids

All plasmids used in this study are listed in Table 1. The promoter regions of PRM1 (from −461 to +3) and PRM4 (from −453 to +3) were amplified from chromosomal DNA using primers PRM1-1 (CCGGATCCTCAGAAGTCTGCTG) and PRM1-2 (CCAAACTTCATATTACGTCAGGTT), and primers PRM4-1 (CCGGATCCTCAGAAGTCTGCTG) and PRM4-2 (CCAAACTTCATATTACGTCAGGTT), digested with BamHI and HindIII, and then cloned into the BamHI and HindIII sites of Yep367 [29]. The three Ste12-binding sites in the PRM1 promoter (from −181 to −175, from −170 to −164, and from −159 to −153) were changed from TGTTC to ATAAATT by a two-step site-directed mutagenesis. First, two PCR products were generated using primers PRM1-1 and PRM1-5 (ACGCACTTCAATTATTTATGTATAATTTATTATGTATTACCCGGACTC) and primers PRM1-2 and PRM1-6 (ACCGACTTCATATTATCTCTATACGTCAGGTT) using amplification as template. In a second PCR reaction the mutated PRM1 promoter was amplified using these PCR products as templates and primers PRM1-1 and PRM1-2. The resulting PCR product was digested with BamHI and HindIII, and then cloned into the BamHI and HindIII sites of Yep367. The mutations were confirmed by DNA sequencing.

2.3. Quantitative mating assays

3 × 10⁶ exponentially growing cells of each mating type were mixed and collected on nitrocellulose filters. The filters were placed on YPD plates for 5 h at 30 °C. Filters were then suspended in water and serial dilutions were plated on selective medium to determine the number of diploids. Mating efficiency was calculated as the percentage of input cells that formed diploids.

2.4. β-Galactosidase assays

Densities of cell cultures were measured by optical density at 600 nm (A₆₀₀). 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 MgSO₄, 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 Na₂CO₃. 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 × (A₄₂₀ - (1.75 × A₅₅₀))/reaction time (min) × culture volume (ml) × A₆₀₀.

3. Results

The zinc cluster proteins Ecm22, Upc2, Sut1 and Sut2 all play important roles in sterol uptake and filamentation [10]. Since Sut1 and Sut2 also regulate mating [25], we tested whether this is the case for Ecm22 and Upc2, too. Deletion of either ECM22 or UPC2 had no effect on mating efficiency, whereas cells lacking both genes displayed a strong mating defect (Fig. 1A). Thus, Ecm22 and Upc2 combine both an important and hitherto unknown role in mating.

We next wanted to know how Ecm22 and Upc2 regulate mating. Both proteins control the expression of genes involved in sterol biosynthesis such as of ERG3, ERG11 and NCP1 [12–14], and sterol import including AUS1, DAN1 and PDR11 [13,20,23]. Ecm22/Upc2-dependent regulation of expression of these genes is not only important for sterol homeostasis but also for other processes such as filamentation and the hyphomycotic shock response [14,30]. However, ERG3, ERG11, NCP1, AUS1, DAN1 and PDR11 levels did not change in response to pheromone treatment (data not shown). It therefore seems that Ecm22 and Upc2 regulate mating through different target genes.

It has been reported that cells carrying the constitutively active upc2-1 allele express PRM1 and PRM4 at increased levels [13]. Prm1 and Prm4 have been identified as transmembrane proteins that are strongly upregulated in response to pheromone [31,32]. A role in membrane fusion during mating has been well established for Prm1 [37]. In contrast, almost nothing is known about Prm4. We therefore examined whether Prm4 is actually involved in mating. Like PRM1 [32], PRM4 is required for efficient mating (Fig. 1B).

We next wanted to know whether Ecm22 and Upc2 mediate mating through regulation of PRM1 and PRM4 expression. PRM1 and PRM4 were both expressed at low levels in exponentially growing cells (Fig. 2A and B). This expression was not affected by deletion of either ECM22 or UPC2 but markedly reduced in the ecm22 upc2 double mutant (Fig. 2A and B). These data suggest that both Ecm22 and Upc2 play an important role in the control of basal expression of PRM1 and PRM4.

As mentioned above, PRM1 and PRM4 expression was strongly upregulated in response to pheromone (Fig. 3A and B) [31,32]. PRM1 and PRM4 induction in ECM22 and UPC2 single deletion strains was comparable to the wild type, in contrast to ecm22 upc2Δ cells in which PRM1 and PRM4 induction was considerably reduced (from 14.5- to 3.7-fold for PRM1 and from 7.3- to 2-fold for PRM4) (Fig. 3A and B). Thus, Ecm22 and Upc2 are required for both basal and pheromone-induced transcription of PRM1 and PRM4.

### Table 1

<table>
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<th>Strain</th>
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<td>Yep367</td>
<td>Δ his, LEU2, lacZ</td>
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The fact that induced PRM1 and PRM4 expression was strongly reduced but not completely abolished in the ecm22 upc2 double mutant (Fig. 3A and B) suggests that other factors also control the transcription of PRM1 and PRM4. The transcription factor Ste12 plays a leading role in the regulation of mating gene expression [4–6]. It binds to pheromone-responsive elements (PREs) in the promoter region of its targets [38,39]. Three consensus Ste12-binding sites [38,39] can be found in the PRM1 promoter (Fig. 4A) but to our knowledge it has not been tested whether Ste12 really regulates PRM1 expression. STE12 deletion resulted in a massive increase of transcription through these three sites. Basal expression from the mutated PRM1 promoter following pheromone treatment was not detectable (Fig. 3A). Taken together, these data suggest that Ecm22/Upc2 and Ste12 regulate PRM1 expression independently of each other.

4. Discussion

The transcription factors Ecm22 and Upc2 have an important and well established role in sterol biosynthesis and sterol uptake [10]. Here, we show that Ecm22 and Upc2 also regulate mating.
Deletion of either ECM22 or UPC2 alone has no effect on mating efficiency or expression of mating genes, whereas cells lacking both genes exhibit strong phenotypes. This suggests that the related proteins Ecm22 and Upc2 have overlapping functions in mating. Such a Ecm22-Upc2 redundancy has previously been shown for other processes such as filamentation [14].

Sterol biosynthesis genes are among the most important targets of Ecm22 and Upc2 [10]. However, expression of sterol biosynthesis genes does not change in response to pheromone (data not shown). This is in line with observations that while sterol composition is not affected by deletions or overexpression. Furthermore, no Ste12 deletion or Ste12 overexpression increases levels of Prm4 [10].

We suggest that Ecm22 and Upc2 mediate mating through induction of PRM1 and PRM4. In this study, we show that Ecm22 and Upc2 play an important role in basal and pheromone-induced transcription of PRM1 and PRM4. The role of Prm1 in membrane fusion during mating has been well characterized [32–37]. Unfortunately, very little is known about Prm4. Since Prm4 is strongly upregulated in response to pheromone and because it contains a predicted transmembrane domain it is a good mating factor candidate [31,32]. Here, we show here that Prm4 like Prm1 is indeed required for efficient mating. Ecm22 and Upc2 also play a role in filamentation, another differentiation process [14]. However, deletion of PRM1 or PRM4 does not affect filamentous growth (data not shown). Furthermore, unlike target genes of Ecm22 and Upc2 that are involved in filamentation [14,19], PRM1 and PRM4 expression does not change during filamentation (data not shown). These observations suggest that Ecm22 and Upc2 regulate PRM1 and PRM4 specifically in mating and not during filamentation.

Around 200 genes are induced in response to pheromone, and almost all mating genes seem to be regulated through the transcription factor Ste12 [6]. However, here we show that PRM4 expression is regulated by Ecm22 and Upc2, and PRM4 levels are not affected by STE12 deletion or overexpression. Furthermore, no Ste12-binding sites could be identified in the PRM4 promoter (data not shown). Thus, it seems unlikely that Ste12 controls PRM4 transcription. Instead, basal and pheromone-induced PRM4 expression is regulated by Ecm22 and Upc2. For PRM1 we could show that its basal expression and pheromone induction is under control of Ste12, Ecm22 and Upc2. Mutation of Ste12-binding sites

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in the PRM1 promoter or simultaneous deletion of ECM22 and UPC2 both markedly reduce PRM1 expression. In the absence of ECM22, UPC2 and Ste12 sites in the PRM1 promoter, PRM1 expression is no longer detectable. This additive effect suggests that Ecm22/Upc2 and Ste12 regulate PRM1 through independent pathways. It remains unknown how Ecm22 and Upc2 are activated in response to pheromone. Very little is known about Ecm22 regulation. Upc2 is regulated through sterol concentration and at the transcriptional level [10]. Upc2 acts as a sterol sensor [23,47,48]. When sterol levels drop, Upc2 translocates from the cytoplasm to the nucleus where it controls gene expression. However, it is unlikely that this is a mechanism for Upc2 activation during mating because sterol levels do not change following pheromone treatment [46]. Control of UPC2 transcription is another important regulatory mechanism. Upc2 stimulates its own expression [13,20] and UPC2 expression is also regulated by Sut1 and Sut2 [14]. However, transcriptional regulation can also be ruled out since expression of UPC2 and ECM22 is not affected by pheromone treatment (data not shown). During mating Ecm22 and Upc2 therefore seem to be regulated through a different yet unknown mechanism.

In conclusion, we have established that Ecm22 and Upc2 are novel mating factors that seem to act independently of Ste12 during mating Ecm22 and Upc2 are regulated during mating because their targets have, and how Ecm22 and Upc2 are regulated during mating.

Conflicts of interest

The author declares no conflicts of interest.

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Transparency document

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