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The repressor Rgt1 and the cAMP-dependent protein kinases control the expression of the *SUC2* gene in *Saccharomyces cerevisiae*

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ABSTRACT

Background: A low level of glucose is required for maximal transcription of the *SUC2* gene in *Saccharomyces cerevisiae*. Although the repressor Rgt1 binds the *SUC2* promoter in gel-shift assays, it has been reported that Rgt1 has only minimal effects on *SUC2* expression. Rgt1 acts together with Mth1 to repress the *HXT* genes encoding glucose transporters, and the release of Rgt1 from some *HXT* promoters requires cAMP-dependent protein kinase (PKA) activity.
Methods: The genes *RGT1* and *MTH1* have been disrupted and the *SUC2* promoter modified in several *S. cerevisiae* backgrounds. Yeast cells were grown in different carbon sources in the presence or absence of 0.1 or 2% glucose, and invertase was assayed in whole cells.
Results: Galactose, glycerol or ethanol hindered invertase induction by low glucose, but lactate did not. During growth in lactate, deletion of *RGT1* or *MTH1* caused a marked increase in invertase levels, and elimination of the Rgt1-binding site in the *SUC2* promoter caused also invertase induction. PKA activity decreased invertase levels in cells growing in lactate, and increased them during growth in lactate + 0.1% glucose.
Conclusions: The low level of expression of *SUC2* in the absence of glucose is mainly due to repression by the Rgt1-Mth1 complex. Repression is dependent on PKA activity, but not on any specific Tpk isoenzyme.
General significance: The results show that previously overlooked regulatory elements, such as Rgt1 and Tpks, participate in the control of *SUC2* expression in *S.cerevisiae*.

1. Introduction

The transcriptional profile of the yeast *Saccharomyces cerevisiae* is strongly affected by glucose, the expression levels of about 40% of its genes being up- or down-regulated when glucose becomes available to yeast cells growing on non-fermentable carbon sources [1,2]. Among the genes regulated by glucose *SUC2*, encoding invertase, has been considered a model to study the mechanisms underlying the repression caused by glucose [3,4]. The elements responsible for the repressing effect of high glucose (2%) on *SUC2* have been thoroughly investigated and the role of many regulatory proteins clearly established [5–9]. Although *SUC2* is repressed by 2% glucose, it requires a low concentration of glucose (below 0.2%) to be induced [10]. A similar pattern of expression had been described for genes such as *HXT2* or *HXT4*, which encode glucose transporters [11], and it has been shown that in the presence of low glucose the DNA-binding protein Rgt1 is removed from the promoters of the *HXT* genes, relieving its repressing effect [12].

Although Rgt1 can bind to the *SUC2* promoter at a consensus TNNTCCG site [13], it has been reported that in a *rgt1* mutant *SUC2* expression remains low in the absence of glucose [10]. Moreover, *SUC2* was not identified among the significant physiological targets of Rgt1 [14]. These results suggested that the activation of the transcription of *SUC2* and of the *HXT* genes by a low concentration of glucose is mediated by different mechanisms. We noticed, however, that in previous studies expression of *SUC2* in the absence of glucose was measured in cells grown on glycerol or galactose [10] and this could have influenced the results, since glycerol and galactose are able to repress *SUC2* (J.M. Gancedo preliminary experiments). We have, therefore, reexamined a possible role for Rgt1 in the control of *SUC2* expression, using a carbon source that does not repress this gene. Expression of *SUC2* has been assessed by measuring secreted invertase. To investigate whether Rgt1 has a direct effect on the *SUC2* promoter, or may also act indirectly, we compared invertase levels in mutants lacking either Rgt1 or the Rgt1-binding site in the *SUC2* promoter. We evaluated also the role of Mth1 on invertase expression, since the interaction of Mth1 with Rgt1 allows its binding to DNA [15].

As it has been shown that the cAMP-dependent protein kinases are required to relieve glucose repression of genes *HXT1* and *HXT3* by Rgt1 [12], we examined also whether these kinases are needed for the induction of *SUC2* by low glucose.

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Table 1
Strains used in this work.

Strain	Relevant genotype	Source/reference
CJM534	*MAT***a** ade2-*1 his3-11,15 trp1 ura3-52*	This laboratory
CJM561	*MAT***a** *ade2-1*	This laboratory
CJM922	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1 ura3-52 rgt1::LEU2*	This study
CJM926	*MAT***a** *SUC2-33 ade2-1 his3-11,15 trp1 ura3-52*	This study
CLF61	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1 ura3-52 mth1::TRP1*	[21]
MB23	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1 ura3-52 tpk2::HIS3 tpk3::URA3*	[22]
MB13	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1 ura3-52 tpk1::LEU2 tpk3::URA3*	[22]
MB12	*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1 ura3-52 tpk1::LEU2 tpk2::HIS3*	[22]
CJM553	*MAT***a** *ade2-1 his3-11,15 trp1 msn2::HIS3 msn4::TRP1*	This laboratory
CJM549	*MAT***a** *ade2-1 his3-11,15 trp1 ura3-52 msn2::HIS3 msn4::TRP1 tpk1::URA3 tpk2::HIS3 tpk3::TRP1*	This laboratory
CJM564	*MATα ade2-1 yak1::KanMX*	This laboratory
CJM1066	*MAT***a** *ade2-1 his3-11,15 trp1 ura3-52 msn2::HIS3 msn4::TRP1 tpk1::URA3 tpk2::HIS3 tpk3::TRP1 rgt1::LEU2*	This laboratory
W *yak1 tpk1/2/3*	*MATα ade2-1 his3-11,15 leu2-3,112 trp1 ura3-52 tpk1::URA3 tpk2::HIS3 tpk3::TRP1 yak1::LEU2*	[23]
CJM1083	*MATα ade2-1 his3-11,15 leu2-3,112 trp1 ura3-52 tpk1::URA3 tpk2::HIS3 tpk3::TRP1 yak1::LEU2 rgt1::ADE2*	This laboratory
CJM319	*MATα ade2-1 his3-11,15 trp1 ura3-52 hxk2::LEU2*	This laboratory
DBY1315	*MATα leu2-3,112 lys2-801 ura3-52*	[24]
CJM866	*MATα leu2-3,112 lys2-801 ura3-52 rgt1::LEU2*	This study
CJM991	*MATα SUC2-33 leu2-3,112 lys2-801 ura3-52*	This study
CJM957	*MATα leu2-3,112 lys2-801 ura3-52 mth1::URA3*	This study
MSY230	*MAT***a** *his4-912δ lys2-128δ leu2Δ1 ura3-52*	[19]
CJM868	*MAT***a** *his4-912δ lys2-128δ leu2Δ1 ura3-52 rgt1::LEU2*	This study
MSY233	*MAT***a** *SUC2-33 his4-912δ lys2-128δ leu2Δ1 ura3-52*	[19]
CJM956	*MAT***a** *his4-912δ lys2-128δ leu2Δ1 ura3-52 mth1::URA3*	This study

2. Materials and Methods

2.1. Yeast strains

Yeast strains used in this study are listed in Table 1. Strains CJM534 and CJM561 were derived from strain W303-1A [16], strains CJM553 and CJM549 from strains W*msn2msn4* [17] and W303 *tpk1/2/3 msn2msn4* [18], respectively, and strain CJM564 from *yak1::KanMX* (gift from C. Brocard) by substituting in the original strains the mutated genes (*leu2, his3, trp1, ura3*) by their wild-type alleles, as needed. In strains CJM922, CJM866, CJM868 and CJM1066, *RGT1* was interrupted by substituting the *Eco*RV$_{1026}$-*Eco*RV$_{2766}$ sequence of the ORF by the *LEU2* gene. In strain CJM1083, *RGT1* was interrupted by substituting the same sequence by the *ADE2* gene. In strains CJM957 and CJM956, *MTH1* was interrupted by substituting the *Afl*II$_{-131}$-*Nco*1$_{1376}$ sequence of the ORF by the *URA3* gene. To replace the wild-type *SUC2* gene by the *SUC2-33* allele, with modifications in the -442 to -431 sequence of the promoter [19], we proceeded as follows. The *SUC2* gene from strains CJM534 and DBY1315 was interrupted from *Nco*1$_{-384}$ to *Nco*1$_{962}$ with the *URA3* gene. The *SUC2::URA3* strains were transformed with the fragment -534 to 1107 from *SUC2-33* obtained by PCR from strain MSY233 and transformants were selected in plates with 5-fluorouracil [20]. The correct integration was checked by PCR. In strain CJM319 the complete reading frame of *HXK2* and its flanking sequences were replaced by the *LEU2* gene in the W303 background.

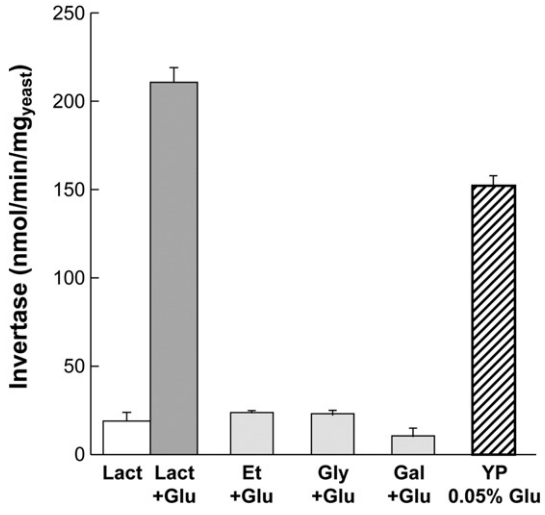

Fig. 1. Invertase activity from yeast cells grown on different carbon sources. *S. cerevisiae* strain CJM561 was grown in SC medium with the indicated carbon sources (Lact, 2% lactate; Glu, 0.1% glucose; Et, 2% ethanol; Gly, 3% glycerol; Gal, 2% galactose). The yeast was also grown in YPD and derepressed for 3 h in YP0.05% glucose. Invertase was measured as described in Materials and Methods, values are averages (± SD of the sample) of three independent cultures.

2.2. Culture conditions and enzymatic measurements

The yeasts were grown in a synthetic medium (SC dropout) [25] with the necessary supplements at a final concentration of 20 mg/l and with different carbon sources (2% lactate, 2% ethanol, 3% glycerol or 2% galactose) in the absence or presence of glucose (0.1% or 2%) and collected at the mid-log phase of growth. Invertase was tested as [26] using whole cells and measuring the glucose formed in the reaction with hexokinase and glucose-6P dehydrogenase; activity is expressed as nanomol/min/mg yeast (wet weight).

3. Results

3.1. Induction of invertase by low glucose in yeast growing in a synthetic medium

Two different transcripts originate from the *SUC2* gene, one of them encoding a constitutive cytoplasmic invertase, and the second one encoding a periplasmic invertase, whose levels are regulated by glucose [3]. We have tested invertase in whole cells, measuring therefore only the periplasmic, regulated form. To examine factors which affect the induction of invertase by low glucose, a prerequisite is to identify a carbon source which does not cause repression of invertase. Therefore we compared invertase levels in yeasts grown on different carbon sources in the presence of 0.1% glucose. Since the yeast extract used to prepare rich media contains some glucose, we used a synthetic medium for these experiments. In addition, we measured also invertase in yeast cells grown on YPD and incubated for a few hours in YP0.05% glucose, as this has been the standard method to determine derepressed levels of invertase [27].

As shown in Fig. 1, a low concentration of glucose (0.1%) induced high levels of invertase when added to lactate medium, similar to those measured after incubation in YP0.05% glucose, while invertase