RESEARCH ARTICLE

Malte Kleinschmidt · Ramona Schulz
Gerhard H. Braus

The yeast *CPC2/ASC1* gene is regulated by the transcription factors Fhl1p and Ifh1p

Received: 2 September 2005 / Revised: 28 November 2005 / Accepted: 4 December 2005 / Published online: 10 January 2006

Abstract *CPC2/ASC1* is one of the most abundantly transcribed genes in *Saccharomyces cerevisiae*. It encodes a ribosome-associated Gβ-like WD protein, which is highly conserved from yeast to man. Here, we show that *CPC2* transcription depends on the carbon source and is induced during utilization of the sugar glucose. *CPC2* promoter deletion and insertion analyses identified two upstream activation sequence elements for *CPC2*, which are required for basal expression and regulation. One of these upstream activation sequence elements has an ATGTACGGATGT motif, which has previously been described as a putative binding site for the forkhead-like transcription factor Fhl1p. Deletion of *FHL1* reduces *CPC2* transcription significantly in presence of glucose, but has no effect when the non-fermentable carbon source ethanol is provided. Increased amounts of the Fhl1p co-regulator Ifh1p induce *CPC2* transcription even when ethanol is utilized. These data suggest that the interaction between Fhl1p and Ifh1p is critical for the regulation of *CPC2* transcription during utilization of different carbon sources.

Keywords *Saccharomyces cerevisiae* · *CPC2/ASC1* · Fhl1 · Ifh1 · Ribosomal protein genes

Introduction

Ribosome biogenesis is a major consumer of cell energy, and therefore, tightly controlled in response to the environmental conditions and the physiological state of the cell. The number of ribosomes is adjusted to the overall protein synthetic capacity of the cell and requires a co-ordinate regulation of all ribosome components.

In *Saccharomyces cerevisiae* transcription of 137 ribosomal protein genes is co-ordinately regulated under different environmental growth conditions (Warner 1999). This co-regulation is controlled by the conserved RAS/protein kinase A, TOR and protein kinase C signalling pathways as response to the growth conditions (Warner 1999). During the nutritional up-shift from a non-fermentable carbon source, such as ethanol, to a fermentable carbon source, such as glucose, transcription of ribosomal protein genes is strongly induced (Herruer et al. 1987; Kief and Warner 1981; Kraakman et al. 1993). In contrast, several environmental insults, such as heat shock, amino acid starvation, nitrogen limitation or osmotic stress, lead to down-regulation of ribosomal protein genes (Causton et al. 2001; Gasch et al. 2000; Natarajan et al. 2001).

The *CPC2* (also known as *ASC1*) gene of *S. cerevisiae* encodes a highly conserved Gβ-like WD-protein of 319 amino acids (Chantrel et al. 1998; Hoffmann et al. 1999) and is a homologue of the *cpc-2* gene of *Neurospora crassa* (Müller et al. 1995), *CPC2* of *Schizosaccharomyces pombe* (McLeod et al. 2000) and of the human RACK1 encoding a receptor of activated protein kinase C (Ron et al. 1994), respectively. The open reading frame of *CPC2* is interrupted by an intron between the corresponding amino acids 179 and 180. This intron is located close to the 3′-end, which is unusual in comparison to other introns in yeast. It has been characterized as containing the U24 small nucleolar RNA (*SNR24*) coding region, which is required for site-specific 2′-*o*-methylation of rRNA (Kiss-Laszlo et al. 1996; Qu et al. 1995). Genome-wide expression analyses described the *CPC2* open reading frame as a highly transcribed gene (Velculescu et al. 1997), finally resulting in an estimated 330,000 Cpc2p molecules per cell (Ghaemmaghami et al. 2003; Velculescu et al. 1997). This high transcription rate was also observed

Communicated by P. Sunnerhagen

M. Kleinschmidt · R. Schulz · G. H. Braus (✉)
Institute of Microbiology and Genetics, Georg-August-University, Grisebachstrasse 8, 37077 Göttingen, Germany
E-mail: gbraus@gwdg.de
Tel.: +49-551-393771
Fax: +49-551-393820

when cells were starved for amino acids (Hoffmann et al. 1999).

Polysome profile analyses and mass-spectrometry identified an association of Cpc2p to the 40S-ribosomal subunit (Chantrel et al. 1998; Gerbasi et al. 2004; Link et al. 1999). Thereby, Cpc2p seems to be present at a 1:1 ratio with ribosomal proteins. In addition to this ribosome bound form, a non-ribosome bound form of Cpc2p was identified (Baum et al. 2004). In cells in a stationary growth phase, the non-ribosome bound form of Cpc2p seems to increase suggesting that association of Cpc2p to the 40S-ribosomes probably depends on the yeast cell activity. Recent experiments showed an increased translation efficiency of specific mRNAs in the absence of Cpc2p. These data suggest a regulatory role for Cpc2p in translation (Gerbasi et al. 2004). A possible role of Cpc2p in translation has also been suggested for the corresponding proteins Cpc2 in *S. pombe* (Shor et al. 2003) and the human RACK1 (Ceci et al. 2003). In human cells, RACK1 acts as signalling platform for several signalling molecules and recruits activated protein kinase C to the ribosome to regulate translation in response to stimuli (Ceci et al. 2003; Nilsson et al. 2004).

Genome-wide chromatin immunoprecipitation (ChIP) analyses revealed that the forkhead-like transcription factor Fhl1p binds to nearly all promoters of yeast ribosomal protein genes (Lee et al. 2002). Fhl1p was originally identified as a multicopy suppressor of RNA polymerase III mutations (Hermann-Le Denmat et al. 1994). It has a domain similar to the forkhead DNA-binding domain found in the developmental forkhead protein of *Drosophila melanogaster* and in the HNF-3 family of hepatocyte mammalian transcription factors. Loss of *FHL1* results in a slow-growth phenotype and in a defect in 35S-rRNA processing. Overexpression of the essential gene *IFH1* (interacting with forkhead) suppresses the slow-growth phenotype of *fhl1* deletion yeast cells (Cherel and Thuriaux 1995). Recent studies found that both Fhl1p and Ifh1p are required for a tight co-regulation of ribosomal protein genes (Lee et al. 2002; Martin et al. 2004; Rudra et al. 2005; Schawalder et al. 2004; Wade et al. 2004). It was shown that Ifh1p is recruited through the forkhead-associated domain of Fhl1p to ribosomal protein gene promoters to induce transcription. The level of Ifh1p association to ribosomal gene promoters depends on environmental conditions and thus, it is one key regulatory step for expression of ribosomal protein genes. Beside ribosomal protein genes, translation factors or other ribosome-associated proteins require a distinct regulation in response to environmental stimuli to ensure an efficient translation of mRNAs.

In this work we show that transcription of *CPC2* is stimulated in glucose-grown cells compared to ethanol-grown cells. The two key regulators of ribosomal proteins Fh1p and Ifh1p are involved in regulation of this transcriptional induction. These data suggest that *CPC2* and ribosomal protein genes are co-ordinately regulated under utilization of different carbon sources.

Materials and methods

Yeast strains and growth conditions

Saccharomyces cerevisiae strains used in this work are listed in Table 1 and are derivates of S288c. For construction of yeast strains RH3094 to RH3101 and RH3269 to RH3274, each carrying a single integration of a *CYC1–lacZ* reporter, plasmids pME2598 to pME2605 were linearized by *Stu*I and chromosomally integrated at the *URA3* locus of RH1168, YPH500 or MW667, respectively. Yeast strains RH3102, RH3261 and RH3262 carrying single *CPC2–lacZ::URA3* reporter cassettes were constructed by integration of plasmid pME2614 at the *URA3* locus. Yeast strains RH3103 to RH13111 and RH3259 carrying individual *cpc2-lacZ* deletion constructs were obtained by *Stu*I digestion and chromosomal integration of plasmids pME2615 to pME2623 and pME2931 at the *URA3* locus. The yeast strain RH3260 (*CPC2*–GFP) was created by PCR-based C-terminal tagging of chromosomal *CPC2* ORF (Knop et al. 1999). Transformations were carried out using the lithium-acetate yeast transformation method (Ito et al. 1983). All integrations were confirmed by Southern hybridization analysis (Ausubel et al. 1993). For measurement of *CPC2* transcript expression, yeast strains were cultivated at 30°C in liquid synthetic minimal medium (YNB) containing appropriate supplements. Cultures were diluted into fresh YNB medium with 2% glucose or 2% ethanol as a carbon source and cultivated for 4 h before assaying enzymatic activities, isolation of total RNAs and fluorescence microscopy, respectively. Functionality of the *CPC2*–GFP fusion construct was tested at 15°C on YEPD medium with 2% agar.

Plasmids

All constructed plasmids are listed in Table 2. Plasmids pME2598 to pME2605 were obtained by amplification and integration of individual 75–150 bp of *CPC2* promoter fragments into pLG669Z (Guarente and Ptashne 1981) using a *Xho*I restriction site introduced at the 5′-end of the PCR primers. For construction of *cpc2–lacZ* reporter plasmids pME2614 to pME2623 and pME2931, 600 bp upstream of the *CPC2* translation start site were amplified and cloned as a *Xho*I fragment into pBluescript II SK +/−. A deletion set of the *CPC2* promoter was generated by whole-vector PCR excluding individual *CPC2* promoter sequences of 1/2, 75 or 150 bp. After religation by using an introduced *Bgl*II restriction site, individual *cpc2-lacZ* deletion constructs were amplified and cloned into YIp356R (Myers et al. 1986) using the *Bam*HI/*Kpn*I restriction sites introduced at the 5′-end of PCR primers. The plasmid pME2932 was constructed by amplification of the *IFH1* ‘open reading frame’ (ORF)

Table 1 *Saccharomyces cerevisiae* strains used in this work

Strain	Genotype	Reference
RH1168	*MATa, ura3-52, leu2-3*	Our collection
RH2419	*MATa, ura3-52, leu2-3, trp1, cpc2::LEU2*	Hoffmann et al. (1999)
RH3094 to RH3100	*MATa, ura3-52, leu2-3, URA3::pCPC2-CYC1-lacZ*	This work
RH3101	*MATa, ura3-52, leu2-3, URA3::CYC1-lacZ*	This work
RH3102	*MATa, ura3-52, leu2-3, URA3::CPC2-lacZ*	This work
RH3103 to RH3110	*MATa, ura3-52, leu2-3, URA3::pcpc2-lacZ*	This work
RH3111	*MATa, ura3-52, leu2-3, URA3::lacZ*	This work
RH3259	*MATa, ura3-52, leu2-3, URA3::pcpc2-lacZ*	This work
RH3260	*MATa, ura3-52, leu2-3, CPC2-GFP::KanR*	This work
YPH500	*MATα, ura3-52, leu2-1, ade2-101, trp1-Δ1, lys2-801, his3-Δ200*	Sikorski and Hieter (1989)
MW667	*MATα, ura3-52, ade2-101, trp1-Δ1, lys2-801, his3-Δ200, fhl1-Δ1::HIS3*	Hermann-Le Denmat et al. (1994)
RH3261	*MATα, ura3-52, ade2-101, trp1-Δ1, lys2-801, his3-Δ200, fhl1::HIS3, fhl1-Δ1::HIS3, URA3::CPC2-lacZ*	This work
RH3262	*MATα, ura3-52, leu2-1, ade2-101, trp1-Δ1, lys2-801, his3-Δ200, URA3::CPC2-lacZ*	This work
RH3269	*MATα, ura3-52, leu2-1, ade2-101, trp1-Δ1, lys2-801, his3-Δ200 URA3::pCPC2*$_{-375/-225}$*- CYC1-lacZ*	This work
RH3270	*MATα, ura3-52, leu2-1, ade2-101, trp1-Δ1, lys2-801, his3-Δ200 URA3::pCPC2*$_{-300/-150}$*- CYC1-lacZ*	This work
RH3271	*MATα, ura3-52, leu2-1, ade2-101, trp1-Δ1, lys2-801, his3-Δ200, URA3::CYC1-lacZ*	This work
RH3272	*MATα, ura3-52, ade2-101, trp1-Δ1, lys2-801, his3-Δ200, fhl1-Δ1::HIS3, URA3::pCPC2*$_{-375/-225}$*- CYC1-lacZ*	This work
RH3273	*MATα, ura3-52, ade2-101, trp1-Δ1, lys2-801, his3-Δ200, fhl1-Δ1::HIS3, URA3::pCPC2*$_{-300/-150}$*- CYC1-lacZ*	This work
RH3274	*MATα, ura3-52, ade2-101, trp1-Δ1, lys2-801, his3-Δ200, fhl1-Δ1::HIS3, URA3::CYC1-lacZ*	This work

using primers containing *Spe*I/*Xho*I restriction sites at the 5′-end and integration into pRS425prom. (Mumberg et al. 1994).

Northern hybridization analysis

Total RNAs from yeast were isolated following the protocol described by Cross and Tinkelenberg (Cross and Tinkelenberg 1991). RNAs were separated on 1.4% agarose gel containing 3% formaldehyde and transferred onto nylon membranes by capillarblotting. Gene-specific probes were ^{32}P-radiolabelled with the MBI Fermentas HexaLable™ DNA Labelling Kit. Hybridization signals were quantified using a BAS-1500 Phosphor-Imaging scanner (Fuji, Tokyo, Japan).

β-galactosidase assay

Assays were performed with yeast extracts derived from cultures grown in liquid medium. Specific β-galactosidase activities were normalized to total protein contents (Bradford 1976) in each extract and equalized ($OD_{415} \times 1.7$)/(0.0045 × protein concentration × extract volume × time) (Rose and Botstein 1983).

GFP fluorescence microscopy

The *S. cerevisae* strain RH3260 was grown in liquid synthetic minimal medium (YNB + Ura, Leu) with either 2% glucose or 2% ethanol as a carbon source. One millilitre cells of the cultures were harvested by

Table 2 Plasmids used in this work

Plasmid	Description	Reference
pLI4	10 kb vector, *CYC1-lacZ, URA3, bla*	Guarente and Ptashne (1981)
pME2598 to pME2604	75 or 150 bp *CPC2* promoter sequence elements cloned into pLI4	This work
YIp356R	6.9 kb vector, MCS, *lacZ, URA3, bla*	Myers et al. (1986)
pME2614	600 bp *CPC2* promoter fragment in YIp356R	This work
pME2615 to pME2623	75 or 150 bp *CPC2* promoter deletions in pME2614	This work
pME2931	12 bp *CPC2* promoter deletion in pME2614	This work
pRS425prom.	6.4 kb vector, *MET25*prom, MCS, *CYC1*term, *LEU2, bla*, 2 μm	Mumberg et al. (1994)
pME2932	*IFH1* ORF cloned into pRS425prom.	This work

centrifugation and immediately viewed in vivo on a Zeiss Axiovert microscope by either differential interference contrast microscopy (DIC) or fluorescence microscopy using a GFP filter set (AHF Analysentechnik AG, Tübingen, Germany). Cells were photographed using a Xillix Microimager digital camera and the Improvision Openlab software (Improvision, Coventry, UK).

Results

The *CPC2* expression increases twofold in glucose-grown cells compared with ethanol-grown cells

The *CPC2* gene of *S. cerevisiae* encodes a highly conserved 40S-ribosomal subunit-associated protein. Expression analyses of the whole yeast genome reveal for *CPC2* a high transcription rate (Velculescu et al. 1997), finally resulting in an estimated 330,000 Cpc2p molecules per cell (Ghaemmaghami et al. 2003). In spite of this high expression, *CPC2* is not essential under the analysed conditions. Therefore we asked whether this gene is constitutively expressed in response to different carbon sources or distinctly regulated as it has been described for ribosomal protein genes. For instance, expression of ribosomal protein genes is co-ordinately induced during the switch from the carbon source ethanol to the source glucose (Herruer et al. 1987; Kief and Warner 1981; Kraakman et al. 1993).

Northern hybridization experiments were performed to analyse the amount of *CPC2* mRNA in cells when either growing with glucose or with ethanol as carbon source. In addition, the transcript levels of the *RPS26A/B* isogenes, which encode both ribosomal proteins of the 40S-ribosomal subunit, were determined to compare transcription of *CPC2* and ribosomal protein genes under these conditions. Figure 1a shows that transcription of *CPC2* is induced by a factor of 2 under utilization of glucose when compared to ethanol conditions. The mRNA level of the *RPS26A/B* isogenes is 2.5-fold induced in presence of glucose (Fig. 1a), which suggests a co-regulation of *CPC2* and ribosomal protein genes during utilization of glucose.

Specific ß-galactosidase activity assays with the *CPC2–lacZ* reporter strain RH3102 were performed to verify glucose-dependent induction. As shown for the *CPC2* mRNA, specific ß-galactosidase activity of the *CPC2–lacZ* reporter increases by a factor of about 2 in presence of glucose (Fig. 1b). These data confirm that *CPC2* is predominantly induced on a transcriptional level in response to the carbon source glucose.

The GFP open reading frame was chromosomally integrated at the *CPC2* 3′-end by homologous recombination to localize Cpc2p in vivo. The functionality of the Cpc2p–GFP fusion protein was verified by comparison of the growth of RH1168 (*CPC2*), RH2419 (*cpc2*) or RH3260 (*CPC2*–GFP) at 15°C for 5 days (Fig. 1c). Cells of the *cpc2* deletion strain RH2419 (*cpc2*) produce a cold sensitivity growth phenotype, whereas cells expressing the Cpc2–GFP fusion protein grow similarly as cells with the wild-type *CPC2* allele. Fluorescence microscopy studies show the cytoplasmatic localization and strong expression of the Cpc2p–GFP fusion protein under glucose conditions (Fig. 1c). Growth in ethanol results in weak Cpc2p–GFP expression with the same localization in the cytoplasm.

These data show that expression of *CPC2* depends on the carbon source. Utilization of a sugar as glucose induces *CPC2* transcription, which is presumably reflected by an increased amount of Cpc2 protein in the yeast cell.

The yeast *CPC2* promoter comprises two UAS elements, which are required for a distinct transcriptional regulation

A *CPC2* promoter deletion set was cloned to a *lacZ* reporter gene to analyse whether the difference in *CPC2* expression between cells utilizing glucose or ethanol depends on the presence of *cis*-acting elements. The set of the *cpc2–lacZ* promoter deletion constructs spans 600 bp upstream of the *CPC2* initiation codon. All constructs have deletions of 75 or 150 bp in the *CPC2* promoter and overlap at a length of 75 bp (Fig. 2a). The *CPC2–lacZ* fusions carrying promoter deletions were chromosomally integrated at the *URA3* locus to ensure a single copy per cell. A deletion was defined to contain an upstream activation sequence (UAS) when *CPC2* expression was reduced by a factor of 2 and more. An upstream repression sequence (URS) corresponds to at least twofold-enhanced expression.

Figure 2a shows that deletions of the regions −600 to −300 relative to the ATG start codon did not significantly affect specific ß-galactosidase activities under glucose or ethanol conditions. This suggests that the *CPC2* promoter is localized in the region −300 to −1. Deletions of the regions −375 to −225, −300 to −150 and −225 to −75 reduced the specific ß-galactosidase activity to approximately 10% of the full-length *CPC2* promoter activity and abolished the carbon source-dependent *CPC2* regulation. Deletion of the region −150 to −75 had a weak influence on basal activity. This effect was under the setting threshold of twofold variation for an UAS element and did not affect regulation. Therefore the region −300 to −150 is required for basal *CPC2* expression and regulation. The absence of an overlap between deletion constructs −375 to −225 and −225 to −75 suggests the presence of at least two UAS elements in the *CPC2* promoter. A deletion of the first 75 bp upstream of the *CPC2* translational start site increased the specific ß-galactosidase activity by a factor of 4 when ethanol was utilized (Fig. 2a). This data suggest a putative URS element within the *CPC2* promoter for the ethanol-dependent expression.

The ß-galactosidase assays of the *CPC2* promoter deletion constructs fused to *lacZ* showed that the *CPC2* promoter comprises the first 300 bp upstream of the translational start site. Within this promoter two regions

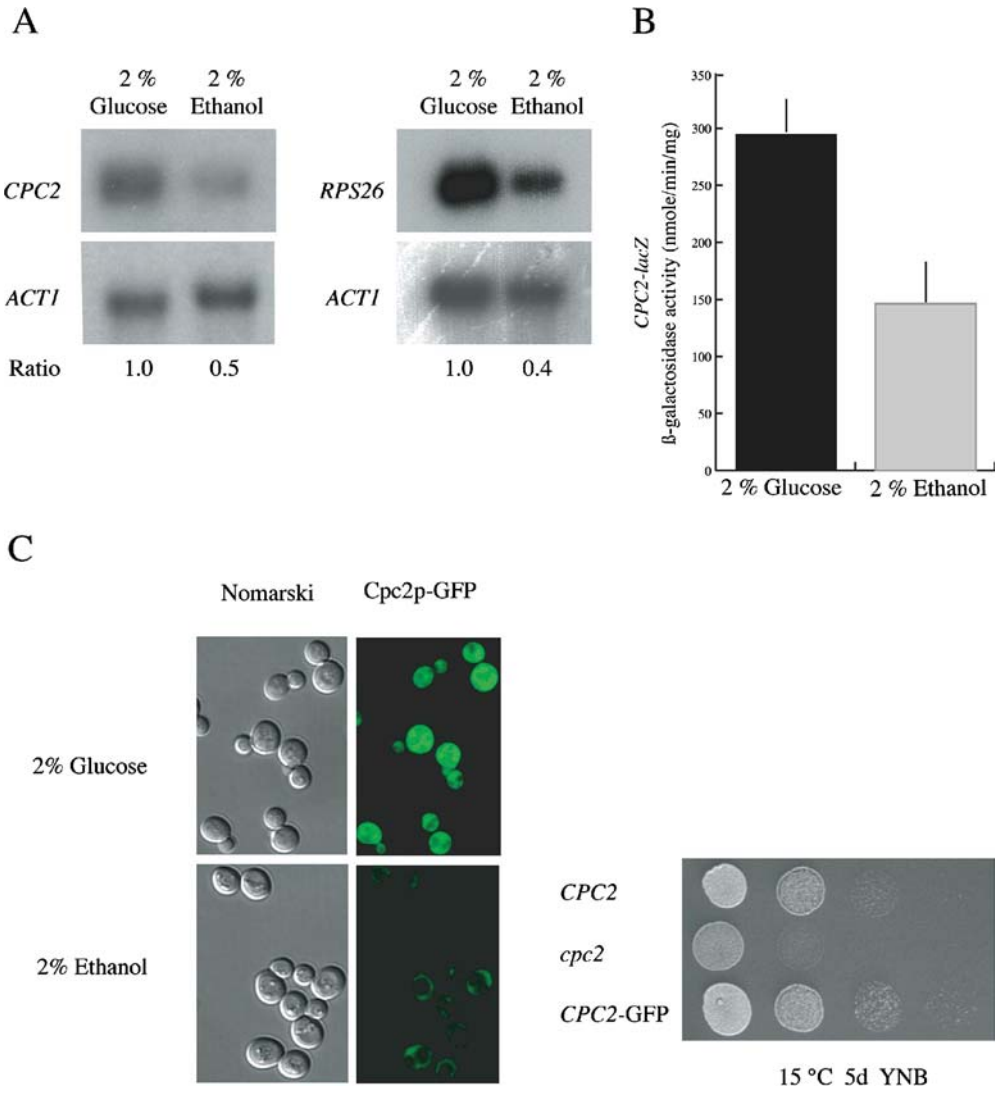

Fig. 1 *CPC2* expression in *Saccharomyces cerevisiae* under utilization of glucose or ethanol as carbon source. The yeast strains RH1168 (*CPC2*), RH3102 (*CPC2*; *URA3*::*CPC2–lacZ*) or RH3260 (*CPC2*–GFP) were cultivated on YNB medium to mid-log phase at 30°C. Cells were sedimented and shifted on YNB medium with 2% glucose or with 2% ethanol for 4 h at 30°C, respectively. **a** 20 µg of total RNA of RH1168 (wt) under glucose or ethanol conditions were used for northern hybridization analyses. *Numbers* indicate relative transcript levels of *CPC2* or *RPS26* in comparison to the internal standard *ACT1*. **b** Expression of the *CPC2–lacZ* reporter construct in the yeast strain RH3102 (*CPC2–lacZ*) was measured after cultivation in glucose (*black bars*) or ethanol (*grey bars*). Units of specific β-galactosidase activities are shown in nanomoles per minutes per milligram. *Bars* depict means of at least three independent measurements. **c** Functional Cpc2–GFP fusion protein was visualized under glucose or ethanol conditions by fluorescence microscopy (*left*). Functionality of the Cpc2–GFP fusion protein was verified by comparing the low-temperature growth defect of RH2419 (*cpc2*) to the yeast strains RH1168 (*CPC2*) or RH3260 (*CPC2*–GFP) on YPD plates at 15°C for 5 days (*right*)

including UAS elements are localized at the position −300 to −225 (UAS_1) and −225 to −150 (UAS_2). Both UAS containing regions elements are necessary for basal *CPC2* expression and in addition for regulation of the promoter.

The *CPC2* promoter −300 to −150 region stimulates activity of the basal yeast *CYC1* promoter

We tested whether single fragments of the *CPC2* promoter are sufficient to stimulate or to repress activity of a basal yeast promoter. *CPC2* promoter fragments of a size of 150 bp that overlap by 75 bp were cloned upstream of the basal *CYC1* promoter, which was fused to the *lacZ* reporter gene (Fig. 2b). All constructs were chromosomally integrated at the *URA3* locus. As shown for the *CPC2* promoter deletion set, insertions carrying fragments of the region −600 to −300 have no effect on the expression of the *CYC1–lacZ* reporter. These data confirm our observation that the *CPC2* promoter ranges from −300 to −1. Fragments carrying the assumed two UAS elements (−375 to −225, −300 to −150 and −225 to −75) are able to stimulate expression of the

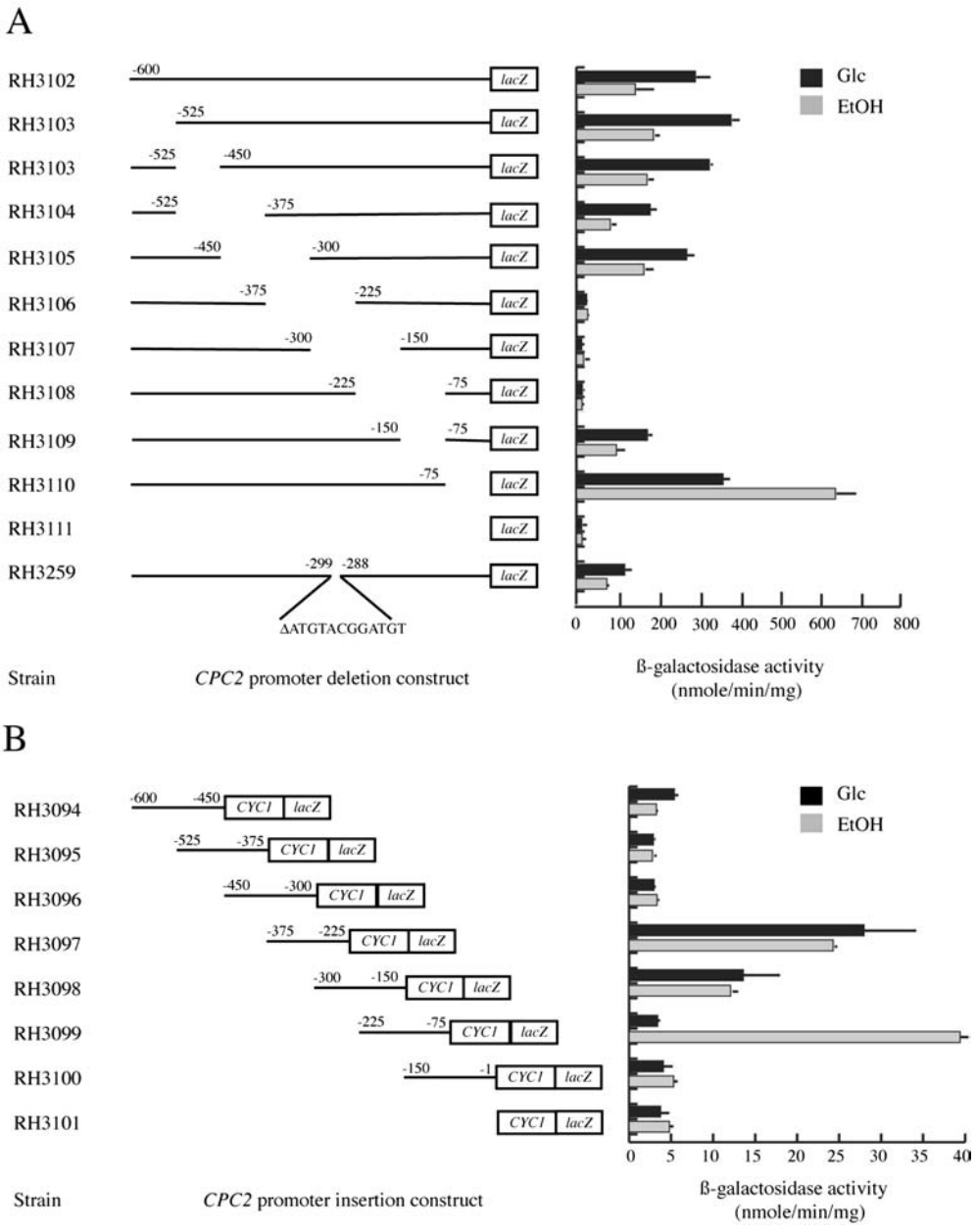

Fig. 2 Expression of a set of deletion or insertion *CPC2–lacZ* reporter constructs in yeast grown under glucose and ethanol conditions. **a** Specific *β*-galactosidase activities were determined in yeast strains RH3102 to RH3110 and RH3259 grown under glucose (*black bars*) and ethanol (*grey bars*) conditions, respectively. Each yeast strain carries an individual p*cpc2–lacZ* promoter deletion construct in addition to a *CPC2* wild-type gene. Deleted segments in the individual constructs are numbered with respect to the translational start site of *CPC2* at position +1. Yeast strain RH3111 served as negative control carrying no *CPC2* promoter element upstream of the *lacZ* reporter. Units of specific *β*-galactosidase activities are shown in nanomoles per minutes per milligram. *Bars* depict means of at least three independent measurements. **b** Expression of individual p*CPC2–CYC1–lacZ* insertion constructs in the yeast strains RH3094 to RH3100 was measured under glucose (*black bars*) or ethanol (*grey bars*) conditions, respectively. Boundaries of insertion are indicated with respect to the translational start site of *CPC2* at position +1. RH3101 was used as negative control strain carrying no insert upstream of the basal *CYC1* promoter of the *CYC1–lacZ* fusion reporter. *Bars* indicate means of specific *β*-galactosidase activities in actual units nanomoles per minute per milligram of least three independent measurements

CYC1–lacZ fusion gene, which supports the existence of at least two UAS in the *CPC2* promoter region (Fig. 2b). Interestingly, insertion of the *CPC2* promoter region −225 to −75 stimulates only the ethanol-dependent expression suggesting either a specific role for this UAS element under ethanol conditions or an artefact as consequence of the *CPC2* and *CYC1* promoter fusion (Fig. 2b). The fragment carrying the putative URS has no effect on the activity of the basal *CYC1* promoter (Fig. 2b). A full-length promoter fragment consisting of 600 bp upstream of the *CPC2* initiation codon did not stimulate expression of the *CYC1–lacZ* reporter (data not shown). These data support that the fusion of *CPC2* and *CYC1* promoters results in an artificial promoter activity.

Measurements of the *CPC2* promoter insertion constructs confirmed the existence of at least two UAS elements for the *CPC2* promoter. The existence of an ethanol-dependent URS element could not be confirmed.

Transcriptional induction of *CPC2* depends on the forkhead transcription factor Fhl1p

Sequence analysis revealed within the UAS_1 region ranging from −300 to −225, a conserved sequence motif at position −299 to −288. This sequence motif consists of the nucleotides ATGTACGGATGT and was proposed in a genome sequence comparison of different

Saccharomyces species as a putative binding site for the transcription factor Fhl1p (Cliften et al. 2003; Kellis et al. 2003). *FHL1* was originally identified as a suppressor of RNA polymerase III mutations (Hermann-Le Denmat et al. 1994). Loss of *FHL1* results in a slow-growth phenotype and in a defect in 35S-rRNA processing. Recent studies revealed a central role for Fhl1p in the regulation of transcription of ribosomal protein genes (Lee et al. 2002; Martin et al. 2004; Rudra et al. 2005; Schawalder et al. 2004; Wade et al. 2004).

An *fhl1* deletion strain (MW667) was tested under glucose and ethanol conditions in Northern hybridization experiments to analyse the impact of Fhl1p on *CPC2* transcription. Figure 3a shows that deletion of *FHL1* reduces *CPC2* transcription by a factor of about 3 under utilization of glucose. Under ethanol conditions the level of *CPC2* mRNA in *fhl1* deletion cells is similar to those in wild-type cells, which suggests that Fhl1p is not required for transcription of *CPC2* in ethanol-grown cells.

Measurements of specific ß-galactosidase activities of a *CPC2–lacZ* reporter in *fhl1* deletion cells confirmed requirement of Fhl1p for *CPC2* expression under glucose conditions (Fig. 3b). Accordingly, cells carrying the *fhl1* deletion showed a twofold reduced specific ß-galactosidase activity in presence of glucose, whereas the basal expression was not impaired.

Northern hybridization experiments and ß-galactosidase assays suggest that the regulator of ribosomal protein genes Fhl1p is required for the glucose-dependent transcriptional induction of *CPC2*. The basal *CPC2* expression under ethanol conditions, however, does not require a functional *FHL1* gene.

The ATGTACGGATGT motif within the *CPC2* promoter region is required for efficient transcription of *CPC2* under glucose and ethanol conditions.

To analyse whether deletion of the putative Fhl1p binding site at position −299 to −289 also influences glucose-dependent transcriptional induction, the specific ß-galactosidase activity of a *cpc2–lacZ* reporter with deleted ATGTACGGATGT motif was assayed. Deletion of ATGTACGGATGT reduces *CPC2* expression by a factor of about 2.5 in presence of glucose (Fig. 2a). Under ethanol conditions deletion of ATGTACGGATGT results in a twofold reduced expression. Since *FHL1* is not required for basal expression of *CPC2*, this suggests that an additional factor binds to the ATGTACGGATGT motif when ethanol as carbon source is utilized.

We further analysed whether *CPC2* promoter fragments carrying the ATGTACGGATGT motif require

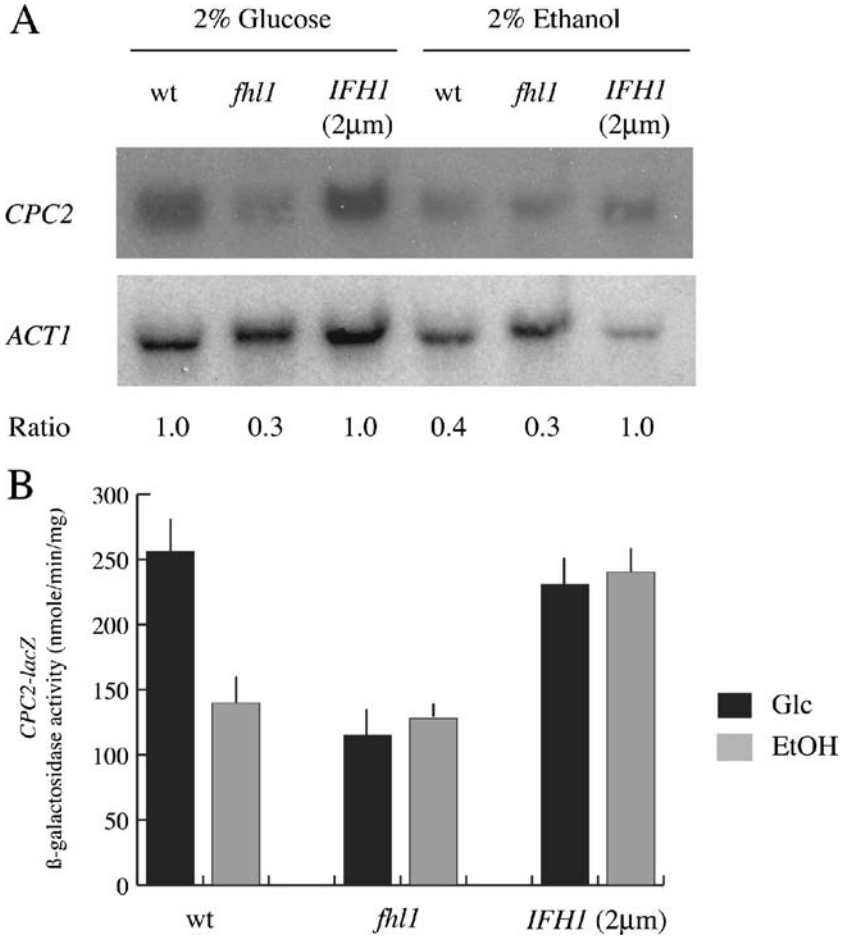

Fig. 3 *CPC2* expression in *fhl1* deletion and *IFH1* overexpression yeast strains under utilization of glucose or ethanol as carbon source. **a** Yeast strains YPH500 (wt), MW667 (*fhl1*) or YPH500 (*IFH1* 2 µm) were cultivated on YNB medium to mid-log phase at 30°C. Cells were sedimented and shifted on YNB medium with 2% glucose or with 2% ethanol for 4 h at 30°C, respectively. Aliquots of total RNA (20–30 µg) from these cultures were used for Northern hybridization analysis. *Numbers* indicate relative transcript levels of *CPC2* to the internal standard *ACT1*. **b** Specific *β*-galactosidase activities of the p*CPC2–lacZ* reporter was assayed in yeast strains RH3261 (wt), RH3262 (*fhl1*) and RH3261 (*IFH1* 2 µm) in presence of 2% glucose (*black bars*) and 2% ethanol (*grey bars*), respectively. *Bars* indicate means of specific *β*-galactosidase activities in nanomoles per minute per milligram of least three independent measurements

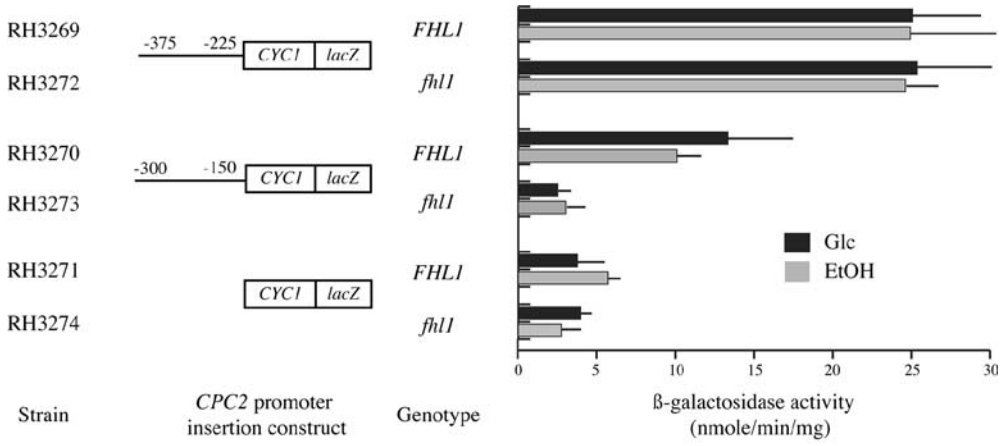

Fig. 4 Expression of *CPC2* promoter insertion constructs in yeast *fhl1* deletion cells under glucose and ethanol conditions. Expression of p*CPC2–CYC1–lacZ* insertion constructs was assayed in the wild-type strains RH3269 and RH3270 or in the *fhl1* deletion strains RH3272 and RH3273 under glucose (*black bars*) or ethanol (*grey bars*) conditions, respectively. Boundaries of insertion are indicated with respect to the translational start site of *CPC2* at position +1. RH3271 and RH3274 were used as negative control strain carrying no insert upstream of the basal *CYC1* promoter of the *CYC1–lacZ* fusion reporter. *Bars* indicate means of specific *β*-galactosidase activities in actual units nanomoles per minute per milligram of least three independent measurements

Fhl1p for stimulation of the basal yeast *CYC1* promoter. Therefore, *CPC2* promoter insertion constructs were integrated into the *fhl1* deletion strain, and the specific *β*-galactosidase activities of these constructs were measured under glucose and ethanol conditions. Figure 4 shows that fragment −375 to −225 enhanced specific *β*-galactosidase activity of the *CYC1–lacZ* reporter in *fhl1* deletion cells. This further supports that an additional factor is able to bind to this region and to affect *CPC2* expression. The *CPC2* promoter insertion construct −300 to −150 requires Fhl1p for activation of the *CYC1–lacZ* reporter under both glucose and ethanol conditions (Fig. 4).

These data suggest that the ATGTACGGATGT motif at position −299 to −288 presumably acts as a specific activation site in combination with Fhl1p as well as a basal element independently of Fhl1p. Measurements of *CPC2* promoter insertion constructs provide indirect evidence of binding of Fhl1p to the promoter region −300 to −150, but not to the region −375 to −225. These different activities of fragments carrying the putative Fhl1p binding site suggest that additional *trans*- or *cis*-acting elements are involved in regulation of *CPC2* transcription.

Overexpression of *IFH1* induces *CPC2* transcription in absence of glucose

The essential protein Ifh1p was first identified as a weak multicopy suppressor of an *fhl1* deletion (Cherel and Thuriaux 1995). Recent studies show that Ifh1p is recruited through Fhl1p to promoters to activate transcription of ribosomal protein genes (Martin et al. 2004; Rudra et al. 2005; Schawalder et al. 2004; Wade et al. 2004). Due to the fact that *IFH1* is essential for cell growth, we transformed yeast strain YPH500 (wt) with plasmid pME2932 (*IFH1*; 2 μm; see Table 2) and tested *IFH1* overexpression in a Northern hybridization experiment for its impact on *CPC2* transcription.

Figure 3a shows that in presence of glucose *IFH1*, overexpression does not affect *CPC2* transcription when compared to wild-type cells. In cells growing with ethanol as a carbon source, overexpression of *IFH1* stimulates transcription of *CPC2* to the level of wild-type cells under glucose conditions.

Measurements of specific ß-galactosidase activities confirmed the Ifh1p-dependent induction of *CPC2* under utilization of ethanol (Fig. 3b). As shown for the *CPC2* mRNA, overexpression of *IFH1* induces the specific ß-galactosidase activity of the *CPC2–lacZ* reporter under ethanol, but does not affect expression under glucose conditions. These data suggest that the amount of Ifh1p is important for transcriptional induction of *CPC2*.

To summarize, transcription of *CPC2* is induced by the carbon source glucose. Two key regulators of ribosomal protein genes, Fhl1p and Ifh1p, are involved in regulation of this process suggesting a putative coordinate regulation of *CPC2* and ribosomal protein genes.

Discussion

The *CPC2* gene has been characterized as abundantly transcribed at levels equivalent to those of ribosomal proteins genes (Velculescu et al. 1997). The high transcription rate results in approximately 330,000 Cpc2 molecules per log-phase cell, which is in the estimated range of 4,500–602,000 molecules of ribosomal proteins per log-phase cell (Ghaemmaghami et al. 2003). In this work, we show that strong transcriptional induction of *CPC2* depends on the carbon source. Under utilization

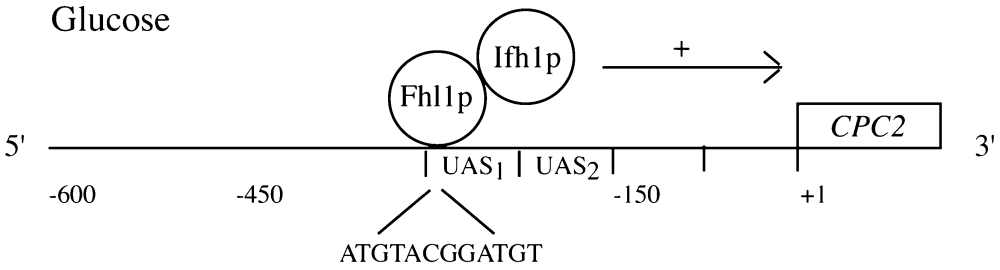

Fig. 5 Scheme for transcriptional induction of *CPC2* in yeast under utilization of the carbon source glucose. Two upstream activation sequence elements (UAS_1 and UAS_2) are shown for the *CPC2* promoter region. *Numbers* indicate positions relative to the translation start site of *CPC2*, which is marked as +1. The ATGTACGGATGT motif represents the presumed binding site for Fhl1p. Fhl1p co-activator Ifh1p stimulates *CPC2* transcription only in the presence of glucose

of the carbon source glucose transcription of *CPC2* is twofold induced when compared to the carbon source ethanol. This is nearly the same factor of induction as we determined for the *RPS26* isogenes as control of genes for the small subunit of the ribosomes. Measurements of the macromolecular composition of *S. cerevisiae* revealed that cells grown on glucose have 2.5-fold more ribosomes than those grown on ethanol (Kief and Warner 1981). A synchronized transcriptional regulation of *CPC2* and ribosomal protein genes has also been identified by transcriptional profiling experiments under different environmental growth conditions (Gasch et al. 2000). All these same characteristics in gene expression of *CPC2* and ribosomal protein genes suggest that Cpc2p is a ribosomal component during utilization of the carbon source glucose. In contrast to many other genes encoding ribosomal proteins, *CPC2* is not present as a gene pair in the yeast genome.

This study also demonstrates that the transcription factor Fhl1p is required for transcriptional induction of *CPC2*. Increased amounts of the transcriptional activator Ifh1p induce *CPC2* transcription also during utilization of the carbon source ethanol. The identification of these two key regulators of ribosomal protein genes suggests for *CPC2* a similar regulatory mechanism as it has been described for ribosomal protein genes (Lee et al. 2002; Martin et al. 2004; Rudra et al. 2005; Schawalder et al. 2004; Wade et al. 2004). Fhl1p presumably binds to the ATGTACGGATGT motif at position −299 to −288 within the *CPC2* promoter region (Fig. 5). This sequence motif has previously been characterized by a genome sequence comparison of different *Saccharomyces* species as a putative binding site for the transcription factor Fhl1p (Cliften et al. 2003; Kellis et al. 2003). Deletion of the sequence ATGTACGGATGT or of *FHL1* reduces transcription of *CPC2* by a factor of about 2.5. These data hint indirectly to an interaction of Fhl1p to the ATGTACGGATGT motif. Genome-wide chromatin immunoprecipitation analyses also confirmed association of Fhl1p with the *CPC2* promoter region (Lee et al. 2002). The association of Fhl1p at the *CPC2* promoter probably requires additional factors. It was shown for several ribosomal protein genes, that in spite of association with the promoter in chromatin immunoprecipitation experiments no binding of purified Fhl1p was detected in vitro by band shift experiments (Rudra et al. 2005). These data suggest that Fhl1p requires other factors or specific chromatin structures to bind gene promoters. Some of these factors might be the RSC chromatin remodelling complex (Angus-Hill et al. 2001), the protein acetylase Esa1p (Reid et al. 2000) or the multifunctional protein Rap1p (Morse 2000; Pina et al. 2003), which have all been described to be involved in regulation of ribosomal protein genes. Interestingly, binding of Rap1p to the *CPC2* promoter was also identified by genome-wide chromatin immunoprecipitation analyses (Lee et al. 2002).

We assume that Fhl1p recruits Ifh1p via its forkhead-associated domain to the *CPC2* promoter when glucose is used as a carbon source (Fig. 5). Thereby, the level of Ifh1p association determines transcriptional induction of *CPC2*. Recent studies with the inhibitor of the TOR pathway rapamycin identified the transcriptional co-repressor Crf1p as an additional factor for regulation of ribosomal protein genes (Martin et al. 2004). In presence of rapamycin, Crf1p enters the nucleus and binds instead of Ifh1p to the forkhead-associated domain of Fhl1p to repress transcription of ribosomal protein genes. Overexpression of Ifh1p suppresses this down-regulation. The fact that also glucose limitation results in a nuclear accumulation of Crf1p suggests that Crf1p is probably involved in carbon source-dependent regulation of *CPC2*.

The increased *CPC2* mRNA level under glucose conditions is also a hint for a transcriptional induction of the highly conserved small nucleolar RNA (*SNR24*), which is located on the *CPC2* intron, and therefore, putatively co-regulated with *CPC2*. *SNR24* is required for site-specific 2′-*o*-methylation of rRNA and is present from yeast to human (Kiss-Laszlo et al. 1996; Qu et al. 1995). Induction of *SNR24* might be important for controlling of pre-RNA folding during ribosome biogenesis.

Beside the ribosome bound form, also a non-ribosome bound form of Cpc2p has been identified by previous studies (Baum et al. 2004). In cells of a stationary culture the non-ribosome bound form of Cpc2p increases, which seems to be dependent on high Cpc2p

levels under such conditions. In *S. pombe* or humans also a non-ribosome form of Cpc2 or RACK1 has been characterized (Ceci et al. 2003; Shor et al. 2003). These data suggest that Cpc2p, beside its function at the ribosomes, also might have additional functions in yeast cells as it has been reported for the homologue proteins of *S. pombe* and mammals. In the fission yeast, Cpc2 is involved in regulation of the cell cycle, actin cytoskeleton organisation or sexual differentiation (Jeong et al. 2004; McLeod et al. 2000; Won et al. 2001). The human RACK1 regulates several processes including cell spreading, recruiting other proteins to focal adhesions or cell-cell contact (McCahill et al. 2002; Nilsson et al. 2004). Tandem affinity purification (TAP) experiments in *S. cerevisiae* also support the suggestion of a multifunctional Cpc2p. Cpc2p was co-purified with 11 different multi-protein complexes with functions in protein synthesis and turnover, transcription/DNA maintenance/chromatin structure, RNA-metabolism, membrane biogenesis and transport (Gavin et al. 2002). Most of these complexes are predominantly localized in the cytoplasm and at the endoplasmatic reticulum, but two complexes involved in transcription/DNA maintenance/chromatin structure and in the RNA metabolism have been reported as localized exclusively in the nucleus. A nuclear localized Cpc2p possibly affects indirectly translation by controlling of ribosome assembly or transport of mRNAs into the cytoplasm.

To summarize, in this work we demonstrate that transcriptional induction of *CPC2* depends on the carbon source glucose. The two key players for a co-ordinated regulation of ribosomal protein genes Fhl1p and Ifh1p are involved in this process. These data suggest that Cpc2p is a ribosomal component and co-regulated to other ribosomal proteins during utilization of different carbon sources.

Acknowledgements We thank Pierre Thuriaux and Hans-Ulrich Mösch for providing yeast strains and plasmids. We are grateful to Katrin Bömeke, Claudia Fischer, Ralph Pries and Oliver Valerius for discussion and critically reading the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie and the Volkswagen-Stiftung.

References

Angus-Hill ML, Schlichter A, Roberts D, Erdjument-Bromage H, Tempst P, Cairns BR (2001) A Rsc3/Rsc30 zinc cluster dimer reveals novel roles for the chromatin remodeler RSC in gene expression and cell cycle control. Mol Cell 7:741–751

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1993) Current protocols in molecular biology. Greene Publishing Associates and Wiley Interscience, New York

Baum S, Bittins M, Frey S, Seedorf M (2004) Asc1p, a WD40-domain containing adaptor protein, is required for the interaction of the RNA-binding protein Scp160p with polysomes. Biochem J 380:823–830

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Causton HC, Ren B, Koh SS, Harbison CT, Kanin E, Jennings EG, Lee TI, True HL, Lander ES, Young RA (2001) Remodeling of yeast genome expression in response to environmental changes. Mol Biol Cell 12:323–337

Ceci M, Gaviraghi C, Gorrini C, Sala LA, Offenhauser N, Marchisio PC, Biffo S (2003) Release of eIF6 (p27BBP) from the 60S subunit allows 80S ribosome assembly. Nature 426:579–584

Chantrel Y, Gaisne M, Lions C, Verdiere J (1998) The transcriptional regulator Hap1p (Cyp1p) is essential for anaerobic or heme-deficient growth of *Saccharomyces cerevisiae*: genetic and molecular characterization of an extragenic suppressor that encodes a WD repeat protein. Genetics 148:559–569

Cherel I, Thuriaux P (1995) The *IFH1* gene product interacts with a fork head protein in *Saccharomyces cerevisiae*. Yeast 11:261–270

Cliften P, Sudarsanam P, Desikan A, Fulton L, Fulton B, Majors J, Waterston R, Cohen BA, Johnston M (2003) Finding functional features in *Saccharomyces* genomes by phylogenetic footprinting. Science 301:71–76

Cross FR, Tinkelenberg AH (1991) A potential positive feedback loop controlling *CLN1* and *CLN2* gene expression at the start of the yeast cell cycle. Cell 65:875–883

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO (2000) Genomic expression programs in the response of yeast cells to environmental changes. Mol Biol Cell 11:4241–4257

Gavin AC, Bosche M, Krause R, Grandi P, Marzioch M, Bauer A, Schultz J, Rick JM, Michon AM, Cruciat CM, Remor M, Hofert C, Schelder M, Brajenovic M, Ruffner H, Merino A, Klein K, Hudak M, Dickson D, Rudi T, Gnau V, Bauch A, Bastuck S, Huhse B, Leutwein C, Heurtier MA, Copley RR, Edelmann A, Querfurth E, Rybin V, Drewes G, Raida M, Bouwmeester T, Bork P, Seraphin B, Kuster B, Neubauer G, Superti-Furga G (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415:141–147

Gerbasi VR, Weaver CM, Hill S, Friedman DB, Link AJ (2004) Yeast Asc1p and mammalian RACK1 are functionally orthologous core 40S ribosomal proteins that repress gene expression. Mol Cell Biol 24:8276–8287

Ghaemmaghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK, Weissman JS (2003) Global analysis of protein expression in yeast. Nature 425:737–741

Guarente L Ptashne M (1981) Fusion of *Escherichia coli lacZ* to the cytochrome c gene of *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 78:2199–2203

Hermann-Le Denmat S, Werner M, Sentenac A, Thuriaux P (1994) Suppression of yeast RNA polymerase III mutations by *FHL1*, a gene coding for a fork head protein involved in rRNA processing. Mol Cell Biol 14:2905–2913

Herruer MH, Mager WH, Woudt LP, Nieuwint RT, Wassenaar GM, Groeneveld P, Planta RJ (1987) Transcriptional control of yeast ribosomal protein synthesis during carbon-source upshift. Nucleic Acids Res 15:10133–10144

Hoffmann B, Mösch HU, Sattlegger E, Barthelmess IB, Hinnebusch A, Braus GH (1999) The WD protein Cpc2p is required for repression of Gcn4 protein activity in yeast in the absence of amino-acid starvation. Mol Microbiol 31:807–822

Ito H, Fukuda Y, Murata K, Kimura A (1983) Transformation of intact yeast cells treated with alkali cations. J Bacteriol 153:163–168

Jeong HT, Oowatari Y, Abe M, Tanaka K, Matsuda H, Kawamukai M (2004) Interaction between a negative regulator (Msa2/Nrd1) and a positive regulator (Cpc2) of sexual differentiation in *Schizosaccharomyces pombe*. Biosci Biotechnol Biochem 68:1621–1626

Kellis M, Patterson N, Endrizzi M, Birren B, Lander ES (2003) Sequencing and comparison of yeast species to identify genes and regulatory elements. Nature 423:241–254

Kief DR, Warner JR (1981) Coordinate control of syntheses of ribosomal ribonucleic acid and ribosomal proteins during

nutritional shift-up in *Saccharomyces cerevisiae*. Mol Cell Biol 1:1007–1015

Kiss-Laszlo Z, Henry Y, Bachellerie JP, Caizergues-Ferrer M, Kiss T (1996) Site-specific ribose methylation of preribosomal RNA: a novel function for small nucleolar RNAs. Cell 85:1077–1088

Knop M, Siegers K, Pereira G, Zachariae W, Winsor B, Nasmyth K, Schiebel E (1999) Epitope tagging of yeast genes using a PCR-based strategy: more tags and improved practical routines. Yeast 15:963–972

Kraakman LS, Griffioen G, Zerp S, Groeneveld P, Thevelein JM, Mager WH, Planta RJ (1993) Growth-related expression of ribosomal protein genes in *Saccharomyces cerevisiae*. Mol Gen Genet 239:196–204

Lee TI, Rinaldi NJ, Robert F, Odom DT, Bar-Joseph Z, Gerber GK, Hannett NM, Harbison CT, Thompson CM, Simon I, Zeitlinger J, Jennings EG, Murray HL, Gordon DB, Ren B, Wyrick JJ, Tagne JB, Volkert TL, Fraenkel E, Gifford DK, Young RA (2002) Transcriptional regulatory networks in *Saccharomyces cerevisiae*. Science 298:799–804

Link AJ, Eng J, Schieltz DM, Carmack E, Mize GJ, Morris DR, Garvik BM, Yates JR 3rd (1999) Direct analysis of protein complexes using mass spectrometry. Nat Biotechnol 17:676–682

Martin DE, Soulard A, Hall MN (2004) TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor *FHL1*. Cell 119:969–979

McCahill A, Warwicker J, Bolger GB, Houslay MD, Yarwood SJ (2002) The RACK1 scaffold protein: a dynamic cog in cell response mechanisms. Mol Pharmacol 62:1261–1273

McLeod M, Shor B. Caporaso A, Wang W, Chen H, Hu L (2000) Cpc2, a fission yeast homologue of mammalian RACK1 protein, interacts with Ran1 (Pat1) kinase to regulate cell cycle progression and meiotic development. Mol Cell Biol 20:4016–4027

Morse RH (2000) RAP, RAP, open up! New wrinkles for RAP1 in yeast. Trends Genet 16:51–53

Müller F, Krüger D, Sattlegger E, Hoffmann B, Ballario P, Kanaan M, Barthelmess IB (1995) The *cpc−2* gene of *Neurospora crassa* encodes a protein entirely composed of WD-repeat segments that is involved in general amino acid control and female fertility. Mol Gen Genet 248:162–173

Mumberg D., Muller R., Funk M. (1994) Regulatable promoters of *Saccharomyces cerevisiae*: comparison of transcriptional activity and their use for heterologous expression. Nucleic Acids Res 22:5767–5768

Myers AM, Tzagoloff A, Kinney DM, Lusty CJ (1986) Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions. Gene 45:299–310

Natarajan K, Meyer MR, Jackson BM, Slade D, Roberts C, Hinnebusch AG, Marton MJ (2001) Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. Mol Cell Biol 21:4347–4368

Nilsson J., Sengupta J., Frank J., Nissen P. (2004) Regulation of eukaryotic translation by the RACK1 protein: a platform for signalling molecules on the ribosome. EMBO Rep 5:1137–1141

Qu LH, Henry Y, Nicoloso M, Michot B, Azum MC, Renalier MH, Caizergues-Ferrer M, Bachellerie JP (1995) U24, a novel intron-encoded small nucleolar RNA with two 12 nt long, phylogenetically conserved complementarities to 28S rRNA. Nucleic Acids Res 23:2669–2676

Pina B, Fernandez-Larrea J, Garcia-Reyero N, Idrissi FZ (2003) The different (sur)faces of Rap1p. Mol Genet Genomics 268:791–798

Reid JL, Iyer VR, Brown PO, Struhl K (2000) Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. Mol Cell 6:1297–1307

Ron D, Chen CH, Caldwell J, Jamieson L, Orr E, Mochly-Rosen D (1994) Cloning of an intracellular receptor for protein kinase C: a homolog of the beta subunit of G proteins. Proc Natl Acad Sci USA 91:839–843

Rose M, Botstein D (1983) Construction and use of gene fusions to *lacZ* (beta-galactosidase) that are expressed in yeast. Methods Enzymol 101:167–180

Rudra D, Zhao Y, Warner JR (2005) Central role of Ifh1p–Fhl1p interaction in the synthesis of yeast ribosomal proteins. EMBO J 24:533–542

Schawalder SB, Kabani M, Howald I, Choudhury U, Werner M, Shore D (2004) Growth-regulated recruitment of the essential yeast ribosomal protein gene activator Ifh1. Nature 432:1058–1061

Shor B, Calaycay J, Rushbrook J, McLeod M (2003) Cpc2/RACK1 is a ribosome-associated protein that promotes efficient translation in *Schizosaccharomyces pombe*. J Biol Chem 278:49119–49128

Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics 122:19–27

Velculescu VE, Zhang L, Zhou W, Vogelstein J, Basrai MA, Bassett DE Jr, Hieter P, Vogelstein B, Kinzler KW (1997) Characterization of the yeast transcriptome. Cell 88:243–251

Wade JT, Hall DB, Struhl K (2004) The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes. Nature 432:1054–1058

Warner JR (1999) The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 24:437–440

Won M, Park SK, Hoe KL, Jang YJ, Chung KS, Kim DU, Kim HB, Yoo HS (2001) Rkp1/Cpc2, a fission yeast RACK1 homolog, is involved in actin cytoskeleton organization through protein kinase C, Pck2, signaling. Biochem Biophys Res Commun 282:10–15