Biochimica et Biophysica Acta 1859 (2016) 405–419

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

The mRNA cap-binding protein Cbc1 is required for high and timely expression of genes by promoting the accumulation of gene-specific activators at promoters

Tianlu Li [a,b], Nikki De Clercq [a,1], Daniel A. Medina [a,b], Elena Garre [a,c], Per Sunnerhagen [c], José E. Pérez-Ortín [a,b], Paula Alepuz [a,b,*]

[a] Departamento de Bioquímica y Biología Molecular, Dr. Moliner 50, Burjassot 46100, Spain
[b] ERI Biotecmed. Universitat de València, Dr. Moliner 50, Burjassot 46100, Spain
[c] Department of Chemistry and Molecular Biology, University of Göteborg, Box 452, S-405 30 Göteborg, Sweden

ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form 8 January 2016
Accepted 12 January 2016
Available online 13 January 2016

Keywords:
Cap-binding protein
Cbc1/Sto1/Cbp80
RNAPII transcription
Osmotic stress
Ribosomal genes

ABSTRACT

The highly conserved *Saccharomyces cerevisiae* cap-binding protein Cbc1/Sto1 binds mRNA co-transcriptionally and acts as a key coordinator of mRNA fate. Recently, Cbc1 has also been implicated in transcription elongation and pre-initiation complex (PIC) formation. Previously, we described Cbc1 to be required for cell growth under osmotic stress and to mediate osmostress-induced translation reprogramming. Here, we observe delayed global transcription kinetics in *cbc1Δ* during osmotic stress that correlates with delayed recruitment of TBP and RNA polymerase II to osmo-induced promoters. Interestingly, we detect an interaction between Cbc1 and the MAPK Hog1, which controls most gene expression changes during osmostress, and observe that deletion of *CBC1* delays the accumulation of the activator complex Hot1–Hog1 at osmostress promoters. Additionally, *CBC1* deletion specifically reduces transcription rates of highly transcribed genes under non-stress conditions, such as ribosomal protein (RP) genes, while having low impact on transcription of weakly expressed genes. For RP genes, we show that recruitment of the specific activator Rap1, and subsequently TBP, to promoters is Cbc1-dependent. Altogether, our results indicate that binding of Cbc1 to the capped mRNAs is necessary for the accumulation of specific activators as well as PIC components at the promoters of genes whose expression requires high and rapid transcription.

1. Introduction

As nascent pre-mRNA emerges from the RNA exit channel of RNA polymerase II (RNAPII) and reaches 22–25 nucleotides during transcription, a characteristic 7-methylguanosine (m^7GpppN) cap structure is co-transcriptionally attached to the 5′ end of the nascent mRNA by three sequential enzymatic steps, which in *Saccharomyces cerevisiae* are catalyzed by Cet1, Ceg1 and Abd1. This cap structure is immediately recognized and bound by the nuclear cap-binding complex (CBC) composed of two proteins of 20 kDa and 80 kDa, termed Cbp20 and Cbp80 respectively (Cbc2 and Cbc1/Sto1, respectively, in yeast) [1–5].

The two subunits of CBC bind the mRNA cap synergistically, as neither has significant affinity to the structure alone [1,2]. The cap-binding pocket resides in Cbp20, however, Cbp80 is required to increase its affinity to the cap structure in which their interaction causes a conformational change in Cbp20. Cbp80 contains a nuclear localization signal (NLS) in its N-terminus, and most likely, the two subunits are co-imported into the nucleus [2]. Cbp20 is found to be unstable as a monomer as its presence is undetectable in Cbp80-depleted extracts [2], and in contrast, Cbp20 does not seem to be required for Cbp80 stability [6].

The importance of CBC to cellular function has been extensively described in mammalian, yeast and plant cells. Although in *S. cerevisiae* the *CBC1* and *CBC2* genes are not essential for cell viability, they have been shown to be required for cell growth and proliferation [7]. In *Arabidopsis thaliana*, disruption of the *CBC* genes is not lethal, but results in developmental delays and abscisic acid (ABA) hypersensitivity [8]. In mammalian cells, siRNA knockdown of CBC results in significant reduction in cell proliferation [9].

Several co- and post-transcriptional mRNA events have been reported to be facilitated by CBC from mammalian to yeast cells. CBC mediates splicing of pre-mRNAs [1,10–13]; participates in transcription termination [6,14,15]; and is involved in mRNA export from the nucleus [2,16,17], although in yeast this function is still unclear [18,19]. The 5′ cap structure and CBC also protects mRNAs from degradation [20,21],

* Corresponding author at: Departamento de Bioquímica y Biología Molecular, Dr. Moliner 50, Burjassot 46100, Spain.
E-mail address: paula.alepuz@uv.es (P. Alepuz).
[1] Present address: Institute for Agricultural and Fisheries Research (ILVO), Brusselsesteenweg 370, 9090 Melle, Belgium.

http://dx.doi.org/10.1016/j.bbagrm.2016.01.002
1874-9399/

although Cbc1 has been found to mediate nuclear and cytoplasmic degradation of some mRNAs [22–25]. Once in the cytoplasm, CBC bound to mRNAs is replaced by the cap-binding protein eIF4E, which allows active translation [26,27]. However, CBC-bound mRNAs can also be translated during the pioneer round of translation [24,25] or even undergo multiple rounds of translation under stress conditions [28–30].

In recent years, several studies have documented a role for CBC in the regulation of gene expression at the transcriptional level. In *S. cerevisiae*, galactose-inducible (*GAL*) genes were shown to require CBC for the binding of pre-initiation complex (PIC) components, such as TBP and RNAPII; however, CBC was dispensable for the binding of the specific *GAL* activator Gal4, the co-activator SAGA, the chromatin remodeling SWI/SNF and the mediator complexes [31]. PIC formation was proposed to occur through the CBC-mediated recruitment of Mot1 to *GAL* genes. Mot1 regulates the dynamic binding of TBP to promoters and has positive or negative effects on transcription in a gene-specific manner [32–37]. Contrastingly, it has been suggested that CBC-mediated recruitment of Mot1 may also function to repress PIC formation on genes that are regulated negatively by Mot1, including some stress-inducible genes [31].

Additionally, CBC has been proposed to be involved in the transition between transcription initiation and elongation. The yeast capping enzymes Cet1, Ceg1 and Abd1 were chromatin immunoprecipitated near the TSS (transcription start site) of several actively transcribed genes, whereas CBC was detected at the 5′ as well as within the gene bodies [31,38,39]. CBC binding to chromatin is likely to occur through its binding to the capped-RNA, as its chromatin association requires both CBC subunits, the methyltransferase Abd1 and RNA [6,31,40]. Abd1 contributes to stable RNAPII occupancy at promoters, and both Abd1 and CBC are important for the recruitment of the RNAPII CTD kinases Bur1 and Ctk1 during elongation [38,41,42]. CBC interacts physically and genetically with Bur1 and Ctk1 and their reduced recruitment in a *cbc* mutant results in lower Ser2 phosphorylation of the RNA pol II CTD in the central and 3′ regions of the genes. Consequently, the trimethylation of histone H3 at Lys36 (H3K36me3), a mark of active transcription, by the methyltransferase Set2 is significantly reduced in a *cbc* mutant [38,42]. A connection between cap formation and transcription elongation has also been documented in mammalian cells. CBC interacts with P-TEFb (positive transcription elongation factor b), which contains Ctk9 (the mammalian homologue of Bur1), and promotes its binding to RNAII CTD to effectively phosphorylate Ser2 in order to mediate transcription elongation of a subset of genes [43]. Despite mounting evidence, other studies indicate that the active role of CBC in transcription is more complex. It has been described that CBC does not play a critical role in promoting the rate or processivity of elongation by RNAPII, as both RNAPII runoff and RNAPII occupancy experiments show no difference between wild type and a mutant harboring a deletion in the *CBP80* gene [6]. Hence, although recent studies have identified a clear role for CBC in transcription, it is still unclear whether this role is general or gene-specific, and which are all the precise mechanisms employed by Cbc1 to regulate transcription.

Transcription patterns are profoundly altered during the response to different external stimuli, such as osmotic stress. Survival in stress conditions requires important changes in yeast physiology (reviewed in [44,45]), as well as rapid and transitory changes in gene expression, which takes place at the transcriptional and post-transcriptional levels ([46–50] and see also reviews [51–54]). Mild osmotic stress alters mRNA levels of hundreds of genes in yeast cells, where most of osmotic stress-induced and part of stress-repressed gene expression are dependent on the mitogen activated protein kinase (MAPK) Hog1, which is homologous to the mammalian p38 MAPK in sequence and function [46,48]. Osmotic stress activates HOG signaling which results in the phosphorylation and nuclear accumulation of Hog1. Subsequently, nuclear Hog1 is recruited to osmo-induced genes by interaction with the stress transcriptional activators Hot1, Msn2, Msn4, Sko1 and Smp1, where each one activates a specific subset of osmotic stress-induced genes [51,52]. Chromatin-bound Hog1 is able to interact with RNAPII to increase its recruitment and activity [55–58].

Previously, our group identified the *cbc1* mutant as sensitive to osmotic stress and showed that Cbc1 depletion delayed the recovery of global translation in response to osmotic stress, and also reduced the proportion of osmo-mRNAs in active translation [29]. In *Arabidopsis*, Cbp20/Cbc2 and Cbp80/Cbc1 have also been described to be involved in osmotic stress tolerance, and a proteomic analysis in *cbc* deletion mutants shows an altered expression of dozens of proteins under osmotic stress [59]. In this study, we investigate the role of *S. cerevisiae* Cbc1 in transcription during the response to hyperosmotic stress using genomic and molecular tools. Determining global transcription rate and promoter binding of several specific and general components of the transcriptional machinery, our results indicate that Cbc1 is necessary for high levels of binding of the specific activator Rap1 to RP genes with and without stress and that Cbc1 interacts with and facilitates the recruitment of the activator complex Hot1–Hog1 to osmostress-responsive genes. Consequently, in the absence of Cbc1, binding of TBP and RNAPII to RP and osmostress genes is reduced, which correlates with reduced transcription levels compared to wild type. Moreover, our genomic study shows Cbc1 to be required for maintaining high levels of transcription of highly transcribed genes under non-stress conditions; meanwhile deletion of Cbc1 has almost no effect on the transcription of weakly transcribed genes. In summary, our results show that stabilization of specific and general transcription factors at promoters by Cbc1 is necessary for keeping high levels of transcription under normal conditions and for high and timely transcription of genes under stress.

2. Materials and methods

2.1. Yeast strains and plasmids

The yeast strains used in this study are listed in Table S1. All strains used were generated in the *S. cerevisiae* haploid strains BY4741 and W303. Null deletion mutants in this study were generated by gene disruption using a polymerase chain reaction (PCR)-based strategy.

To 6HA tag genomic wild type *HOT1*, a PCR-based genomic tagging technique was employed. The plasmid PA171 served as a template for PCR reactions using primers HOT1-1HA and HOT1-2HA (Tables S2 and S3), in which the resulting cassette was transformed into *cbc1Δ* and wild type strains. Transformants were selected in synthetic complete medium lacking histidine to yield strains PAY730 and PAY732 (Table S1).

All plasmids used in this study are listed in Table S2. DNA constructs were generated using conventional PCR, restriction and ligation methods. Otherwise indicated, fusion proteins were expressed from centromeric plasmids and under native promoters.

2.2. Growth conditions and stress

Yeast cells were grown at 30 °C, or otherwise indicated, to mid-log phase in liquid YPD medium (1% yeast extract, 2% peptone, 2% glucose) or synthetic complete media (1.7 g/l yeast nitrogen base (Difco), 5 g/l $(NH_4)_2SO_4$, 20 g/l dextrose and the appropriate drop-out mix (Formedium) lacking the corresponding amino acids to allow selection. For experiments with temperature-sensitive mutants, strains were maintained at the permissive temperature of 23 °C and transferred to the non-permissive temperature of 37 °C during mid-log phase for 1 h. Osmotic stress was performed by adding 0.6 M KCl for the indicated time. Phenotypic analyses were performed by plating serial dilutions of the indicated strains at mid-log phase on YPD plates containing the corresponding salt stress and were then incubated at the indicated temperatures.

2.3. GRO protocol, normalizations and bioinformatic analysis

To determine the transcriptional rate (TR) and the mRNA amount (RA) in the strains BY4741 and *cbc1Δ* mutant, Genomic Run-On (GRO) assay was performed as previously described in [60] with modifications as indicated in [48]. Yeast samples were grown to mid-log phase and osmotic stress was applied by treatment with 0.6 M KCl for 0, 8, 15, 30 and 45 min. Three independent experiments were performed. The macroarray images were quantified using Array Vision software (Imaging Research Inc.). Values that were at least 1.2 times higher than the local background were taken as valid measurements. The TR and RA signals of each macroarray were normalized by those obtained from the same macroarrays hybridized with a random primer genomic DNA label. An average data set for each time point was created using Median Absolute Deviation normalization by ArrayStat software (Imaging Research Inc.). The RA data were adjusted by the concentration of total mRNA per cell as described in [60]. Whole genomic-wide data sets were processed with Microsoft Excel 2007 spreadsheet and the statistical language R [61]. The heatmap cluster was generated with the gplots R-package, and the gene ontology (GO) enrichment in each cluster group was performed using the GOrilla online tool [62]. The redundant GO categories were filtered using the REVIGO online tool [63]. The enrichment p-values were obtained with REVIGO and the adjusted p-values after correction by multiple categories were obtained with Gorilla on line tool that includes the FDR (False Discovery Rate) test for multiple categories correction. Raw and processed data sets are stored in the Gene Expression Omnibus data base (GEO), with the accession number GSE72356.

Yeast genes in Hog1-dependent or independent and in very-rapidly and rapidly induced gene clusters were obtained according to previous GRO data [48]. The classification of yeast genes in five distinct regulons based on their transcriptional dependency on Hot1, Msn2, Msn4, Sko1 and Smp1 was obtained from YEASTRACT database [64] or *Saccharomyces* Genome Database [65], which were then cross-referenced with our GRO data to select the up-regulated genes during osmotic stress treatment. In Fig. 7, the ratio between *cbc1* TR and wild type (wt) TR at time 0 was calculated. Then, under-represented categories were obtained by FATISCAN [66] and filtered by REVIGO [63]. p-Values (Fisher test) were obtained by FATISCAN. In Fig. 9, the 10th quartile of the highest and lowest transcribed genes was determined by sequentially ordering the genes according to their TR in wt cells at time 0.

2.4. Chromatin immunoprecipitation

Immunoprecipitations of RNAPII were performed utilizing the mouse antibody 8WG16 (Convance, Princeton, USA) in combination with Dynabeads Pan Mouse IgG (Life Technologies, Massachusetts, USA). Immunoprecipitations of HA-tagged Hot1 and TBP1 were performed utilizing the rat anti-HA antibody (Roche, Basel, Switzerland) in combination with Dynabeads Protein G (Life Technologies, Massachusetts, USA). Finally, immunoprecipitations of Rap1 were performed utilizing the rabbit anti-Rap1 (y-300) antibody (Santa Cruz, Texas, USA) in combination with Dynabeads Protein A (Life Technologies, Massachusetts, USA). Chromatin immunoprecipitation experiments were performed as previously described [55] with the following modifications: after reversing formaldehyde-mediated cross-linking, samples were treated with Proteinase K (Roche) and DNA was purified using the GeneJET PCR Purification Kit (Fermentas #K0702) according to the manufacturer's instructions. To determine enrichment of DNA regions bound to the proteins of interest, qPCR was performed as described [67] using primers listed in Table S3. QPCR amplification data were normalized using the control primer pair FUS1-1F/FUS1-5, which amplifies a non-coding region upstream of the gene *FUS1*.

2.5. RNA extraction and qPCR analyses

RNA extraction, reverse transcription and quantitative PCR (RT-qPCR) analyses were carried out as previously described [67]. To quantify *STL1*, *GRE3*, *GPD1* and *RPL30* mRNA expression, RT-qPCRs were performed using primers indicated in Table S3. The transcript amounts were normalized against endogenous *ACT1* expression using established primers M1 and M2 (Table S3).

2.6. Western blotting

Western blot analyses were performed as previously described [68]. HA-tagged proteins were detected using the mouse anti-HA antibody (University of Vienna, Austria). To detect endogenous Rap1 and phosphorylated Hog1, rabbit anti-Rap1 [y-300] (Santa Cruz, Texas, USA) and rabbit anti-phospho-p38 (Cell Signaling, Massachusetts, USA) were used, respectively. Total endogenous RNA polymerase II protein expression was detected using the antibody 8WG16 (Convance, Princeton, USA), and phosphorylated RNA polymerase II was detected using anti-RNA polymerase II CTD repeat YSPTSPS (phospho S2) antibody (Abcam, Cambridge, UK). Endogenous Pgk1 and Cdc28 protein expressions were used as loading controls and were detected using mouse anti-Pgk1 (Invitrogen, Carlsbad, USA) and rabbit anti-Cdc2 (Santa Cruz, Texas, USA) antibodies, respectively. Anti-mouse and anti-rabbit HRP-conjugated secondary antibodies were from Promega (Wisconsin, United States). Immunoblots were detected using an ECL Prime kit (GE Healthcare, Buckinghamshire, UK). Band intensities were analyzed using the program ImageQuant TL (GE Healthcare, Buckinghamshire, UK), and quantifications were normalized against the loading control. Figures show representative westerns blots and the averages and standard deviations (SD) were calculated from 2–4 independent experiments.

2.7. M-track analyses of protein interactions

M-track analyses of protein interactions were carried out as previously described [69,70]. To detect methylation signal, immunoblots were incubated with specific mouse anti-me3K9H3 antibody (clone 6F12H4, Millipore, Massachusetts, USA). Anti-me3K9H3 monoclonal antibody was diluted 1/2000 in PBS buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH = 7.4) containing 1% yeast extract and a protease inhibitor cocktail (Roche, Basel, Switzerland). HA and myc tags were detected using anti-HA clone 16B12 (Covance Research Products, Princeton, USA) and anti-myc clone 4A6 (Millipore, Massachusetts, USA), respectively. Band intensities were quantified as described above, and histone methylation signal was normalized against HA expression for each sample, which was then compared to the signal of the control strain without stress. Figures show one representative western blot and the averages and standard deviations (SD) were calculated from two independent experiments.

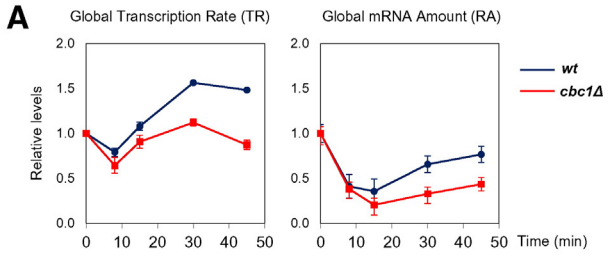
3. Results

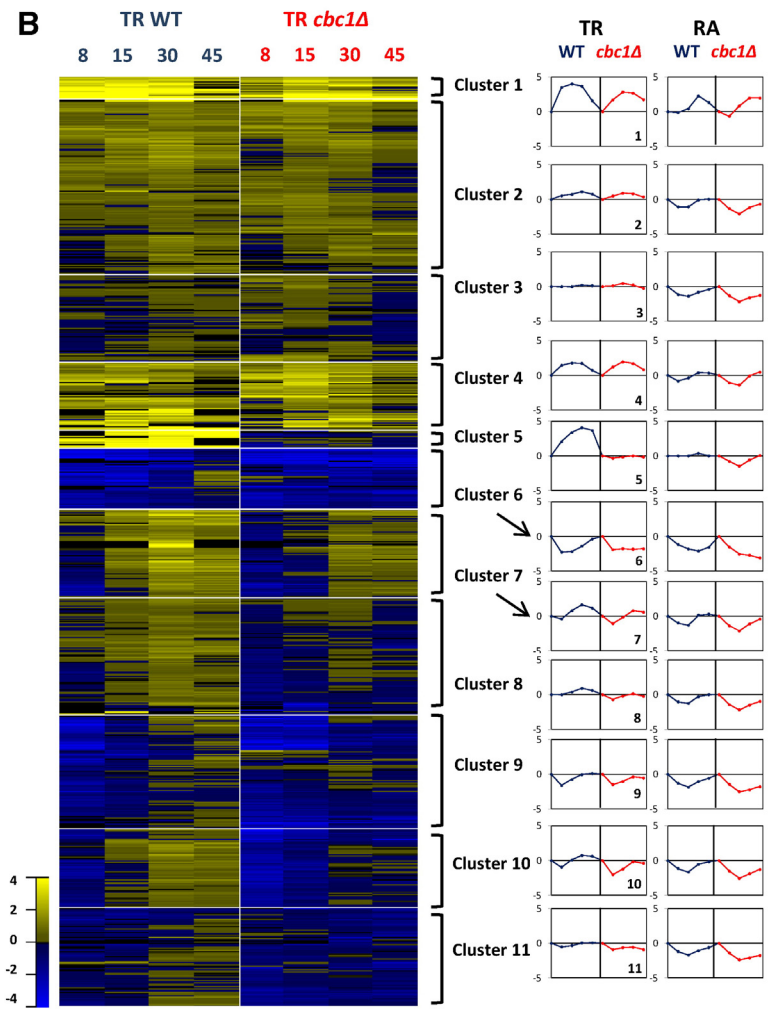
3.1. Deletion of CBC1 results in a deficient global transcriptional response to osmotic stress

In a previous study conducted in our laboratory, we found the *cbc1Δ* mutant to be sensitive to osmotic stress and Cbc1 to be required for reaching a high percentage of osmo-mRNAs associated with polysome-mediated active translation [29]. We also observed that the mRNA amount (RA) induction kinetics of three osmostress induced genes was delayed compared to wild type (wt) kinetics [29]. In order to investigate whether the delay in RA kinetics in response to osmotic stress was general or gene specific and whether this was caused by defects in transcription or mRNA turnover, we utilized the technique Genomic Run-On (GRO) [48,60] with which we evaluated, at the genomic scale, changes in

transcription rate (TR) and RA during osmotic stress provoked by 0.6 M KCl in wt and *cbc1Δ* cells at times 0, 8, 15, 30 and 45 min. In concordance with previous data [48], global TR and RA were observed to rapidly decrease upon osmotic shock and recover during adaptation phase in wt, with global TR recovering earlier than RA (Fig. 1A). However, in *cbc1Δ*, a larger decrease in both TR and RA was observed compared to wt, followed by a slower and reduced recovery, indicating that Cbc1 is required for the reprogramming of global TR and RA following osmotic stress (Fig. 1A).

Next, we analyzed both TR and RA of individual genes and found that the majority of genes changed their TR and RA profiles in *cbc1Δ* compared to wild type (Fig. 1B). All genes analyzed (5868 genes) were then divided into 11 clusters based on similarities in their TR profiles, and a number of functional categories (Gene Ontology, GO) were found to be significantly enriched. The majority of the clusters showed lower TR and RA profiles in *cbc1Δ* compared to wild type during osmotic stress. Many genes that corresponded to stress responses fell under cluster 1, which showed the most rapid and highest TR induction





GO / gene	p-value	Adjusted p-value
response to oxidative stress	-3.0	-0.77
response to abiotic stimulus	-4.3	-0.77
STL1 GPD1 HGI1		
mitochondrial respiratory chain complex	-5.9	-2.15
cellular component organization	-4.3	-0.81
glycogen biosynthetic process	-3.1	-0.57
GRE3		
reproductive process	-4.2	-1.75
cellular developmental process	-3.1	-0.88
RNA processing	-35.6	-32.43
translation	-30.6	-29.29
RPL30, RPL33B		
catabolic process	-5.8	-4.13
glucose metabolic process	-3.6	-1.44
mitochondrial ribosome	-4.0	-0.94
organellar ribosome	-4.0	-0.64
macromolecule biosynthetic process	-5.5	-2.41
RNA metabolic process	-4.7	-2.36
regulation of gene expression	-4.4	-2.35
translational initiation	-3.9	-2.13
No GO Enrichment Found		
DNA strand renaturation	-4.5	-1.63
nucleic acid metabolic process	-4.0	-0.78

Fig. 1. Effect of *CBC1* deletion on changes in TR and RA during the response to osmotic stress. Genomic data were obtained from the median of three independent Genomic Run-On (GRO) experiments normalized as described in the Materials and methods section, where wild type and *cbc1Δ* were treated with 0.6 M KCl for 0, 8, 15, 30 and 45 min. (A) Graphic representation of the medians of global transcription rate (TR) and mRNA amount (RA) for 5868 genes for wild type (blue) and *cbc1Δ* (red) treated with 0.6 M KCl or untreated. Stressed samples were normalized, in each case, against the non-stressed sample of each experimental group. Error bars represent standard deviation (SD) of all analyzed genes. (B) Heat map (left panel) showing the classification of all analyzed genes based on TR, where each lane corresponds to one gene and each column corresponds to the samples treated with 0.6 M KCl for the indicated time for wild type or *cbc1Δ*. Stressed samples were normalized, in each case, against the non-stressed sample, time 0, of each experimental group. Blue represents relative TR repression and yellow represents relative TR induction. The middle panels show graphical representations of the medians of TR and RA of the genes of each cluster for wild type and *cbc1Δ*. Right panel shows significantly enriched GO functional categories of each cluster with the $\log_{10}$ p-value indicated (p-value) and it is also indicated the $\log_{10}$ of adjusted p-values after correction by a FDR test (adjusted p-value). Genes analyzed in this study are written in italics.

upon osmotic stress, which included typical osmostress up-regulated genes, such as *STL1* and *GPD1*, encoding a glycerol/proton symporter and a NADH-dependent glycerol-3-phosphate dehydrogenase, respectively. TR and RA plots of cluster 1 showed a clear delay in *cbc1Δ* compared to wild type (Fig. 1B). For some clusters, such as, 5, 7, 8 and 10, an increase in TR levels during stress was observed in wt and not in *cbc1Δ*. Genes involved in translation, such as those encoding for ribosomal protein (RP) genes, fell in cluster 6, in which a rapid decrease in TR and RA and later recovery was observed in wt; meanwhile no recovery was observed for the same genes in *cbc1Δ* cells (Fig. 1B). In all, it can be concluded that the differences in RA kinetics observed in *cbc1Δ* compared to wild type during osmotic stress are extended to most genes and correlate with delayed and lower TR kinetics.

3.2. The transcriptional function of Cbc1 during osmotic stress extends to Hog1-dependent and Hog1-independent genes and affects all up-regulated transcriptional regulons

The majority of the transcriptional regulation of gene expression during osmotic stress is controlled by the MAP kinase Hog1 [51,54]. Therefore to determine the interdependency between Cbc1 and Hog1, we analyzed the effect of *CBC1* deletion on Hog1-dependent and -independent genes. According to our previous data [48], we selected the clusters of RA-induced (at least 2.5 times increase in RA) and RA-repressed genes (at least 2.5 decrease in RA) which were Hog1-dependent (at least 2-fold higher RA in wt than in *hog1*) or Hog1-independent (less than 2-fold higher RA in wt than in *hog1*). For each of these four groups we then compared the median TR in wt and *cbc1Δ* obtained in our GRO experiment (Fig. 2A). RA induced genes showed a rapid increase in TR in wt that was delayed in *cbc1Δ*; moreover, this delay was observed in both Hog1-dependent and -independent genes (Fig. 2A, upper panel). For RA repressed genes, a rapid decrease in TR was observed which begun to recover after 30 min of 0.6 M KCl treatment and was mostly recovered at 45 min in wt cells; however, no recovery in TR was observed in *cbc1Δ*. Again, this difference in TR kinetics of repressed genes between wt and *cbc1Δ* mutant was independent of Hog1 regulation (Fig. 2A, lower panel). It could therefore be concluded that Cbc1 has roles in the rapid increase of TR of induced genes and in the TR recovery of repressed genes during osmotic stress, and that at least part of its function is independent of Hog1.

Until now, five different transcription factors have been described to be involved in Hog1-dependent up-regulation of genes during osmotic stress [45,51]. We analyzed the TR profiles of genes whose up-regulation are described as Hot1-, Msn2-, Msn4-, Sko1- and Smp1-dependent genes (see the Materials and methods section for further description). As shown in Fig. 2B, for all five transcriptional regulon subsets deletion of *CBC1* resulted in delayed and lower TR kinetics during the stress response. Additionally, osmotic stress-induced genes can be classified into two groups: those induced very rapidly, where their TR reached maximum at 15 min of osmotic stress in wt, and those induced rapidly, where their TR reached maximum at 30 min in wt (Fig. 2C). The TR profiles of both clusters were different for *cbc1Δ* compared to wt, where both showed a delayed TR increase in *cbc1Δ* (Fig. 2C).

Overall, the analyses of TR and RA profiles, utilizing the GRO method, indicate that during osmotic stress Cbc1 plays a role in the celerity of the transcriptional up-regulation of induced and repressed genes, during initial osmotic shock and recovery phase respectively. *CBC1* deletion affects most genes, independent of the role of Hog1 in their regulation, the kinetics of induction and the specific transcription factor responsible for their induction.

3.3. Recruitment of RNA polymerase II to osmo-induced genes is deficient in *cbc1Δ* mutant

Recently, Cbc1 has been involved in the control of transcription through the regulation of the pre-initiation complex (PIC) formation at galactose-inducible (*GAL*) genes [31] and through the recruitment of transcription elongation kinases [38,42]. Therefore, the observed delay in TR increase in *cbc1Δ* could be caused by a defect in transcription initiation or elongation. To evaluate the molecular mechanisms employed by Cbc1 to up-regulate transcription during osmotic stress, we specifically analyzed three osmostress-responsive genes: *STL1* whose induction is dependent on Hog1/Hot1 [55,56,71]; *GPD1*, whose induction is partially HOG-dependent and regulated by the transcription factors Msn2/4 and Hot1 [55,72]; and *GRE3*, which encodes a protein that functions in carbon source metabolism and whose induction is HOG-dependent and regulated by Msn2/4 [73]. As seen in Fig. 3, the TR of *STL1*, *GRE3* and *GPD1* were rapidly induced following osmotic stress in the wt strain. In contrast, a delay was observed in the induction of TR of these genes in *cbc1Δ*. This delay in TR also corresponded to a delay in RA, which could be observed by RT-qPCR data. Furthermore, the RT-qPCR data also showed a slower down-regulation of osmo-mRNA levels at longer times of osmotic stress stimulation in *cbc1Δ* with respect to wt (Fig. 3, left panels, 60 min), which is in concordance with our previous data [29]. Delayed recovery of RA levels after osmotic stress of the ribosomal protein encoding gene *RPL30* was also confirmed by RT-qPCR (Fig. 3, left lower panels). Next, we measured RNAPII occupancy at these genes by chromatin immunoprecipitation (ChIP) experiments, and observed a delayed binding of RNAPII to both promoter and ORF of *STL1* and *GRE3* genes in *cbc1Δ* during osmotic stress (Fig. 3, two right panels). Therefore, it can be concluded that the delayed recruitment of RNAPII to osmostress-genes in turn causes a delay in both TR and RA induction, which may directly contribute to the osmostress-sensitive phenotype observed in *cbc1Δ*. Using western blotting, we evaluated whether the delayed RNAPII recruitment in *cbc1Δ* was due to a change in total and/or active RNAPII protein amount. We observed that there were no significant differences in the ratio of Ser2-phosphorylated/total RNAPII between the mutant and wt (Fig. S1), indicating that the delayed, and sometimes reduced, recruitment of RNAPII to osmostress genes observed in *cbc1Δ* is not due to differences in total or elongating RNAPII amounts in the mutant.

3.4. Activation of transcription by Cbc1 seems to be dependent on Cbc2

Cbc1, together with Cbc2, forms the cap-binding complex (CBC), where the m7GpppN cap-binding pocket resides in the Cbc2 subunit, however, binding of Cbc1 to Cbc2 causes a Cbc2 conformational change that is required for the high affinity binding of the complex to the cap structure [4,74,75]. Therefore, neither CBC subunit alone has significant affinity for the cap [1,2]. We investigated whether the role of Cbc1 in transcription during osmotic stress was dependent on its ability to bind the m7GpppN cap structure, and hence dependent on the presence of Cbc2. Utilizing a *cbc2Δ* deletion mutant, we evaluated its ability to grow under osmotic stress. As observed in Fig. 4A, *cbc2Δ* showed similar growth defects as *cbc1Δ* under both NaCl and KCl stress. Upon evaluation of osmostress-responsive mRNA expression following osmotic stress, we observed a delay in RA induction in *cbc2Δ* compared to wt (Fig. 4B), where the RA profiles were similar to those observed in *cbc1Δ* (Fig. 3). These results suggest that the role of Cbc1 in transcription of osmostress-responsive genes is dependent on its ability to bind the m7GpppN cap structure through Cbc2.

3.5. Cbc1 does not require Mot1, but interacts and facilitates the recruitment of the transcription complex Hot1–Hog1 on osmotic stress induced genes to promote PIC formation

The role of Cbc1 in facilitating RNAPII recruitment at *GAL* genes has been proposed to be mediated through the Cbc1-recruitment of the Mot1 transcription factor [31]. Mot1 has been described to act as a nucleosome remodeler and TBP regulator, and has been shown to induce transcription at TATA-like promoters, including RP genes, and repress transcription at TATA promoters, which encompasses the majority of

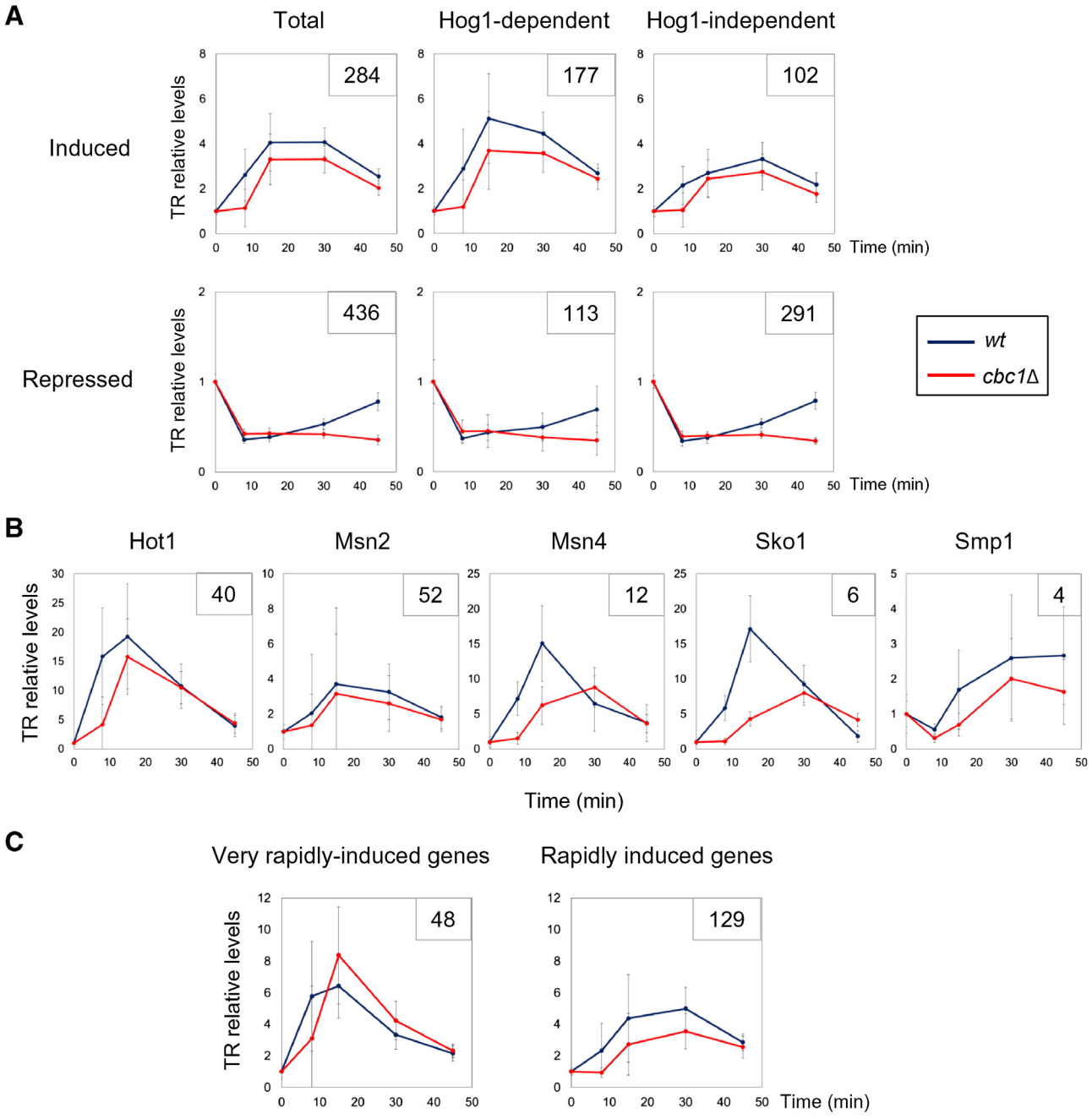


Fig. 2. *CBC1* deletion influences TR and RA kinetics during osmotic stress of up- and down-regulated genes and functions independently of Hog1. Genomic data were obtained for wild type and *cbc1Δ* treated with 0.6 M KCl for 0, 8, 15, 30 and 45 min as indicated in Fig. 1. (A) Graphical representations of the TR medians and standard error (SE) of all induced and repressed genes (left panel). Middle and left panels show clustering of genes that are Hog1-dependent and Hog1-independent, respectively of both up-regulated and down-regulated genes in response to osmotic stress. (B) Graphical representations of the median and SE of up-regulated genes that were divided into five distinct regulons based on their transcriptional dependency on Hot1, Msn2, Msn4, Sko1 and Smp1. (C) Medians and SE of up-regulated genes which are classified as very-rapidly or rapidly induced genes. Genes were classified in each group (indicated in A, B and D) as described in the Materials and methods section. The number of genes in each group is indicated in a box into the graphics.

stress genes, under normal growth conditions ([76,77] and reviewed in [78,79]). To investigate whether Mot1 was involved in regulation of gene induction under osmotic stress, we used a temperature-sensitive *mot1* mutant and evaluated the inductions of the osmo-mRNAs *STL1*, *GRE3* and *GPD1* following Mot1 depletion by incubating at the restrictive temperature of 37 °C, and this was compared with the osmo-mRNA induction profiles in a *cbc1Δ* mutant at the same temperature. As expected [68], the RA kinetic induction profiles in the wt strain were more rapid at 37 °C than at 30 °C (compare Fig. S2 with Fig. 3 and 4B). At 37 °C, the mRNA induction of osmo-mRNAs following osmotic stress was delayed in *cbc1Δ* relative to wt. However, the depletion of Mot1 did not change the induction profiles, suggesting that Mot1, unlike Cbc1, is not required for their rapid transcription following osmotic stress; neither does it seem to function as negative regulator of the expression of these osmo-mRNAs (Fig. S2). Therefore Cbc1 appears to utilize Mot1-independent mechanisms to induce transcription under osmotic stress.

The delay in RNAPII recruitment to osmotic stress induced genes could be caused by a defect in the recruitment of the specific activators, which are required to promote PIC formation and RNAPII binding, although this function has been discarded for Cbc1 in the induction of *GAL* genes since the absence of Cbc1 does not alter the recruitment of the specific activator Gal4 [31]. To check this possibility, we selected the osmotic stress activator Hot1, which plays a critical role in the transcription of ~70 genes

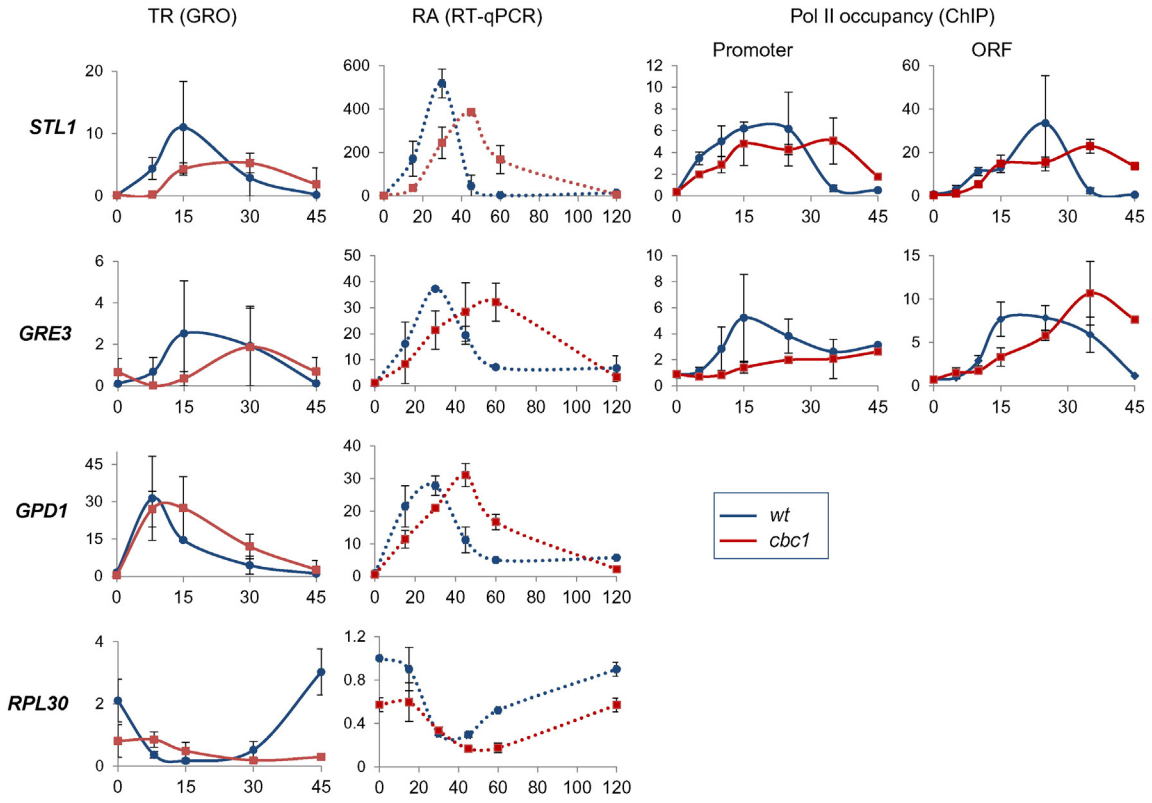


Fig. 3. Recruitment of RNA polymerase II to up-regulated genes is delayed in *cbc1Δ* mutant. The left panel shows averages of TR of genes *STL1*, *GRE3*, *GPD1* and *RPL30* from 3 independent GRO experiments in which wild type and *cbc1Δ* were treated with 0.6 M KCl for 0, 8, 15, 30 and 45 min. The middle panel shows RT-qPCR analyses of mRNA expression of same genes and conditions and for the indicated times of stress. Relative mRNA expression was calculated by first normalizing against the reference gene *ACT1* of each sample and then represented as fold-change relative to wild type non-stress of each experiment. Averages and SD of independent experiments are represented graphically. The two right panels show chromatin immunoprecipitation analysis (ChIP) of RNAPII recruitment to the promoters and ORFs of *STL1* and *GRE3* in wild type and *cbc1Δ* under 0.6 M KCl stress and non-stress for the indicated times. Immunoprecipitation of RNAPII was performed using the antibody 8WG16. Promoters and ORFs were amplified by qPCR using specific primers, and signals were normalized against a non-coding region upstream of *FUS1*, and then represented as fold-change relative to wild type non-stress of each experiment. Averages and standard deviations of independent experiments are represented graphically. For all graphs, a blue line represents wild type and a red line represents *cbc1Δ*.

during osmotic stress (Yeastract database), and whose specific transcriptional regulon is induced upon stress in a Cbc1-dependent manner (Fig. 2B). Upon osmotic stress, Hot1 is hyperphosphorylated by Hog1 and forms a Hot1–Hog1 complex at osmostress promoters, which promotes PIC formation and reallocates RNAPII from housekeeping genes to Hot1-dependent osmostress-responsive genes [55–57]. Utilizing

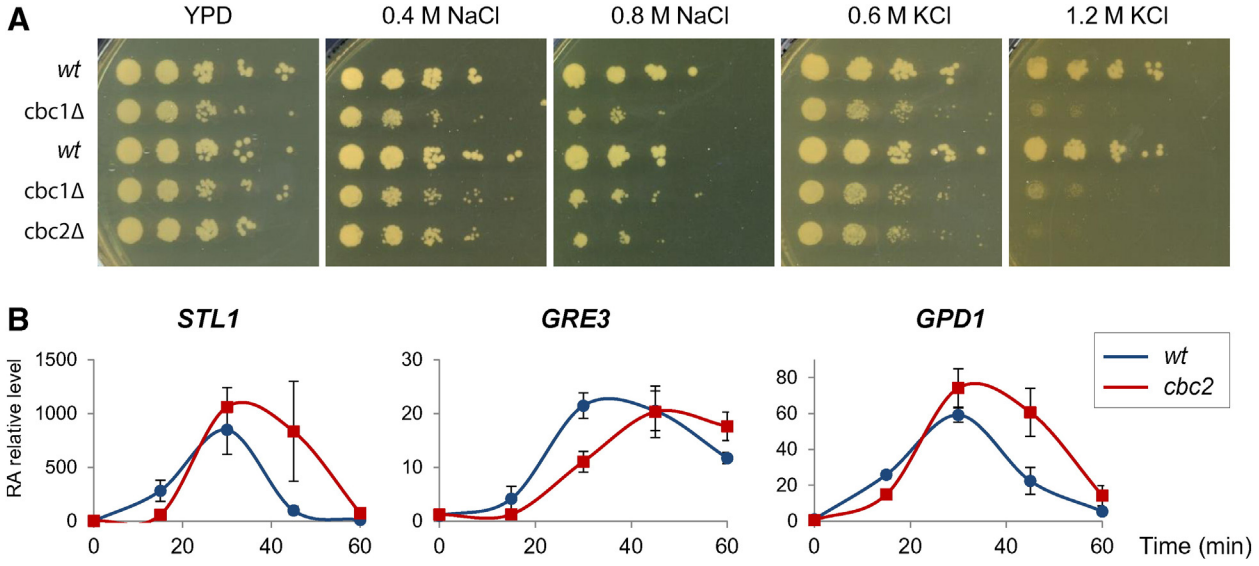


Fig. 4. Cbc2 is necessary for transcription of osmostress genes. (A) Serial dilutions of the indicated yeast strains were grown on YPD agar plates containing the indicated salt concentrations. Plates were incubated until growth of wt strain was observed. (B) RT-qPCR analyses of *STL1*, *GRE3*, and *GPD1* mRNA expression in wild type and *cbc2Δ* (blue and red respectively) during 0.6 M KCl treatment for 60 min show a delay in osmo-RNA induction in the mutant. Relative mRNA expression was calculated by first normalizing against the reference gene *ACT1* of each sample and then as fold-change of wild type non-stress (time 0) of each experiment. Averages and SD of independent experiments are represented graphically.

ChIP analyses, we evaluated Hot1 occupancy at the *STL1*, *GPD1* and *HGI1* osmostress induced promoters in the presence and absence of Cbc1, and observed that at all three promoters Hot1 enrichment was significantly reduced and delayed in *cbc1Δ* following osmotic stress (Fig. 5A). This defective Hot1 binding was coupled with reduced and delayed TBP occupancy at the three promoters in *cbc1Δ* (Fig. 5A).

Next, utilizing the previously described M-track method [69,70], we assessed the ability of Cbc1 to interact with the Hot1–Hog1 complex. For

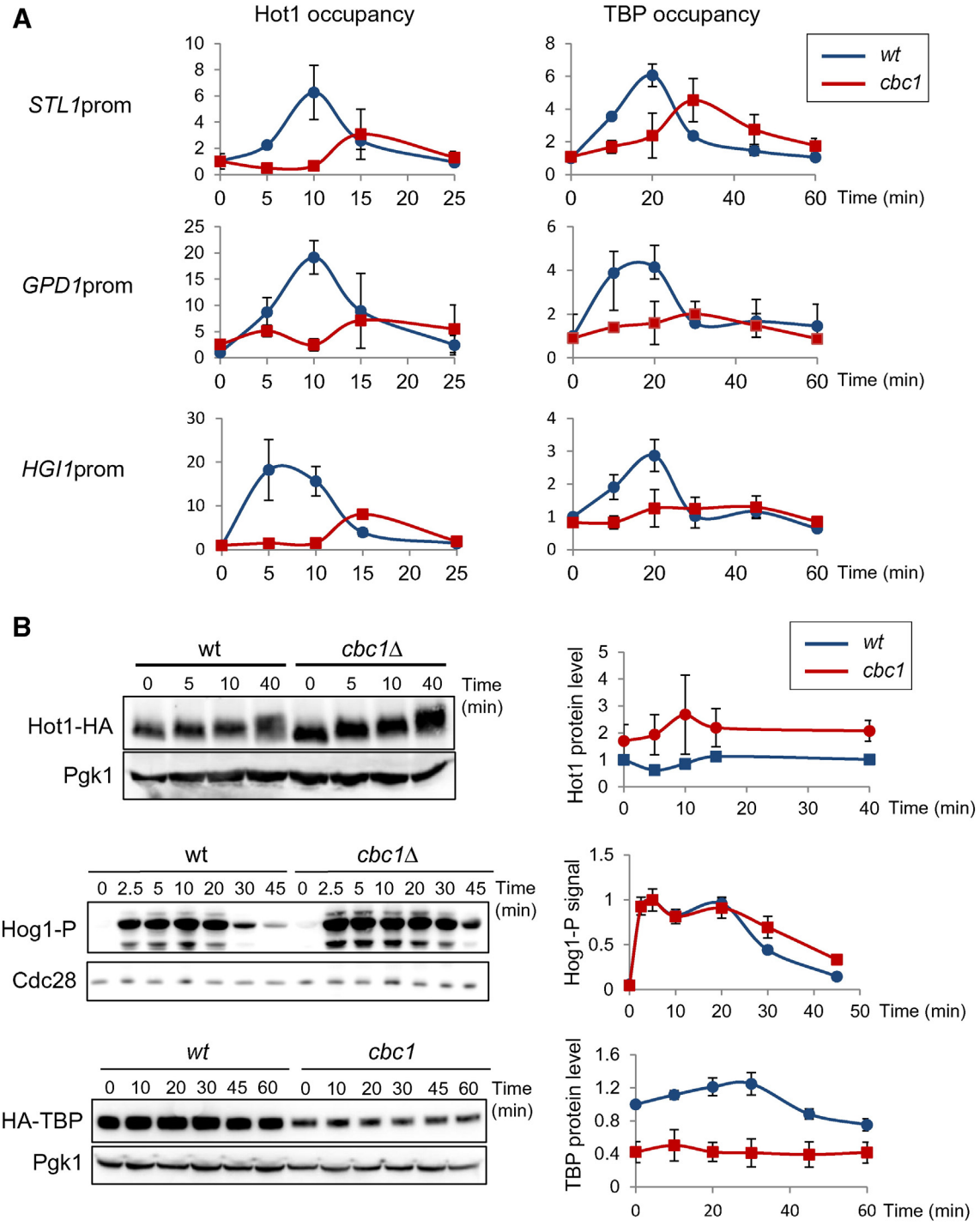


Fig. 5. *CBC1* deletion does not affect rapid signaling upon osmotic stress but delays Hot1 recruitment and PIC formation at osmo-gene promoters and changes Hot1 and TBP protein levels. (A) Graphical representations of ChIP analyses of Hot1 and TBP recruitment to the *STL1*, *GPD1* and *HGI1* promoter in wild type (blue) and *cbc1Δ* (red) during 0.6 M KCl treatment for 25 min. Immunoprecipitations of genomically tagged Hot1-HA and HA-TBP expressed in a centromeric plasmid (PA149) were performed using an anti-HA antibody. Promoter sequence was amplified by RT-qPCR using specific primers, and signals were normalized against a non-coding region upstream of *FUS1*, and then represented as fold-change in respect to wild type non-stress of each experiment. Averages and SD of independent experiments are represented graphically. (B) Left panels show representative western blots depicting Hot1 (probed with anti-HA antibody), phosphorylated Hog1 (probed with anti-phospho p38 antibody) and TBP (probed with anti-HA antibody) protein expressions before and after 0.6 M KCl treatment at the times indicated. Loading controls Pgk1 and Cdc28 were detected utilizing anti-Pgk1 and anti-Cdc2 antibodies, respectively. Right panels show the average and SD of band intensities of at least 3 independent experiments. Band intensities the protein of interest were normalized against the band intensities of loading controls of each sample, and all relative expressions were represented as a fold change relative to wild type non-stress of each experiment.

this experiment, Cbc1 was expressed as a fusion protein containing a hyperactive mutant version of the mouse histone lysine methyltransferase (HKMT) and Hog1 fused with tandem array copies of the histone H3 (H3) epitope. Upon interaction, HKMT methylates H3 which was then detected with a specific antibody by western blotting. Furthermore, Cbc1 and Hog1 fusion proteins were also tagged with myc and HA respectively to allow quantification of fusion protein expressions. As seen in Fig. 6, a methylation signal was detected both before and after osmotic stress, which was 10-fold higher than the background signal, indicating a robust Cbc1–Hog1 interaction. The intensity of the Cbc1–Hog1 methylation signal was similar to other interactions detected by M-track between HOG signaling components [70], but weaker than the strong interaction detected between the Hog1 and its substrate Rck2 (Fig. 6A) [80]. The Cbc1–Hog1 interaction was also tested in a *hog1Δ* mutant strain to avoid competitive binding of endogenous Hog1 to methylase-fused Cbc1. As expected a stronger (3-fold) Cbc1–Hog1 interaction signal was detected in a *hog1Δ* mutant compared to wt (Fig. 6). The M-track signal detected between Cbc1 and Hog1 is a consequence of a direct interaction or of a close proximity between the proteins, although the experiment does not show that the interaction occurs necessarily at the gene promoters.

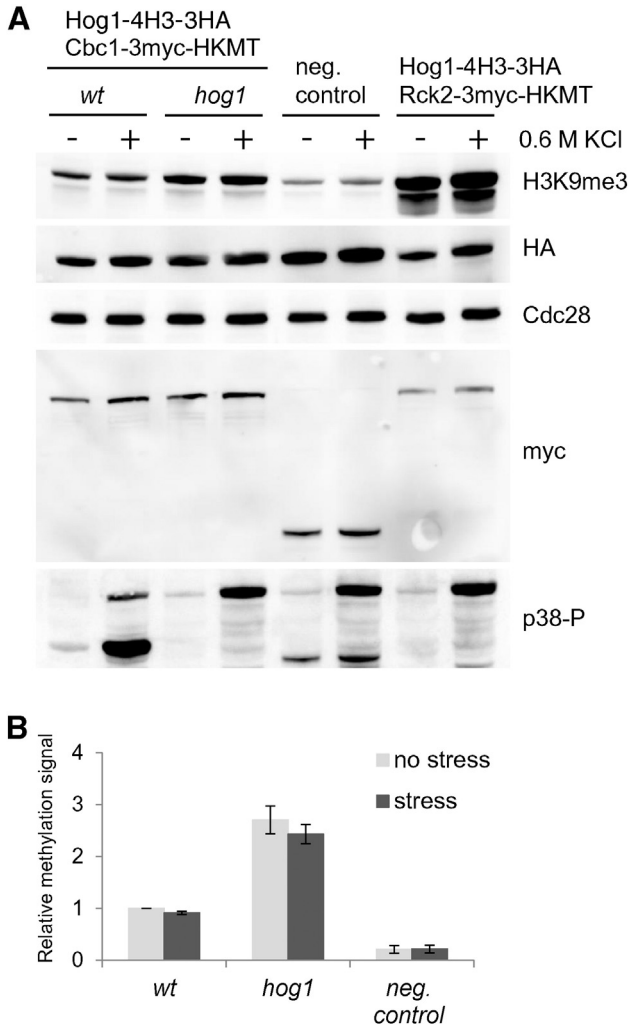


Fig. 6. Cbc1 interacts with Hog1. (A) A representative Western blot showing the interaction of *HOG1–4H3–HA* (CF1E5) and *CBC1–3MYC–HKMT* (P15) detected with anti-methylase antibodies (anti-meK9H3) in the wild type and *hog1Δ* (PAY185) strains in the absence or presence of 0.6 M KCl treatment for 15 min. Negative control corresponds to *hog1Δ* (PAY185) transformed with *HOG1–4H3–HA* (CF1E5) and a plasmid containing only the myc tag and methylase (A187.9 *3MYC–HKMT*). Positive control corresponds to *hog1Δ* (PAY185) transformed with HOG1–4H3–HA (CF1E5) and its substrate *RCK2–3MYC–HKMT* (P12) which were known to interact strongly [105]. Immunoblots were also developed with anti-HA antibody to detect HOG1–4H3–HA expression, as well as probed with anti-Cdc2 to detect the endogenous control Cdc28. Myc-tagged proteins were detected with an anti-myc antibody, and phosphorylated Hog1, to observe HOG signaling, was detected with the anti-phospho p38 antibody. (B) Quantification of the methylation signals normalized against HA signal and compared to the wild type in the absence of stress. The average and standard deviations (SD) of 2 independent experiments are shown.

Altogether, our results suggest that Cbc1 interacts with Hog1 and facilitates the recruitment of the Hot1–Hog1 complex to osmostress promoters, which results in the recruitment of TBP and initiation of transcription by RNAPII of osmostress genes. Therefore, and contrary to results with *GAL* genes [31], Cbc1 does not appear to function through Mot1, but regulates the binding of gene-specific activators to promoters.

3.6. Loss of Cbc1 does not affect activation of osmotic stress signaling, but influences Hog1/Hot1 dephosphorylation and Hot1 and TBP protein levels

The delayed and reduced Hot1 recruitment to osmostress induced genes observed in *cbc1Δ* could be caused by a delayed or deficient osmotic stress signaling. HOG pathway is activated upon osmotic stress, and Hog1 is phosphorylated within the first minutes of stress and then rapidly phosphorylates Hot1 [56,81]. Investigating the phosphorylation kinetics of Hog1 and Hot1 as indicators of a correct signaling during osmotic stress, we observed no delay or reduction in the level of phosphorylation of either protein in *cbc1Δ* mutant during the first minutes of stress (Fig. 5B). Moreover, a significant increase in Hot1 protein was observed in *cbc1Δ*, which indicated that there was no defective osmotic stress signaling activation in *cbc1Δ*, and, additionally, that the reduction in Hot1 binding to osmostress induced promoters cannot be explained by a reduction in Hot1 protein level. On the other hand, for both Hog1 and Hot1, there was an apparent delay in dephosphorylation kinetics during osmotic stress adaption (Fig. 5B). Next, we also checked TBP protein levels in *cbc1Δ* and detected a significant reduction in TBP protein expression in *cbc1Δ* compared to wt before and after osmotic stress (Fig. 5B), and therefore we cannot rule out that the decrease in TBP enrichment at promoters in the absence of Cbc1 was not a consequence of reduced protein expression. Alternatively, it is possible that binding of transcription factors, Hot1, Hog1 and TBP to chromatin could result in altered protein stability and/or dephosphorylation kinetics. In this sense, the stronger phosphorylation signals observed for Hot1 and Hog1 at longer times of osmotic stress exposure correlate with the transcriptional activation still observed at longer times in *cbc1Δ* relative to wild type (Figs. 3 and 4), which suggests that dephosphorylation is related to chromatin binding.

Altogether, our data show that deletion of *CBC1* does not cause a defect in osmostress-activated signaling that could justify the observed delay in Hot1–Hog1 chromatin recruitment, but does cause changes in Hot1 and TBP total protein levels and in the dephosphorylation kinetics of Hot1–Hog1.

3.7. Transcription of ribosomal protein (RP) genes under non-stress and osmotic stress conditions is strongly affected in a cbc1Δ mutant

Our genomic data showed that during osmotic stress most genes, not only induced genes, show transcriptional deregulation in a *cbc1Δ* mutant (Figs. 1 and 2), and data from other authors show a role for Cbc1 in the transcription of several genes in unstressed cells [31,38,42]. Using our genomic data under non-stress conditions (time 0), we compared global TR data between wt and *cbc1Δ*, and, although a good correlation (R-Pearson 0.863) was observed, the slope of the regression line was lower than 1 (slope 0.811) indicating that many genes have lower TR in *cbc1Δ* than in wt (Fig. 7A). A search for GO functional categories in genes that showed lower TR in *cbc1Δ* relative to wild type (Fig. 7A) revealed the group of "ribosome" as the most over-represented (p-value 5.6e−9). To study the specific effect of *CBC1* deletion on RP gene transcription, we calculated the *cbc1Δ*/wt ratio of global TR median obtained in our GRO experiment and compared with the TR ratio for RP genes under

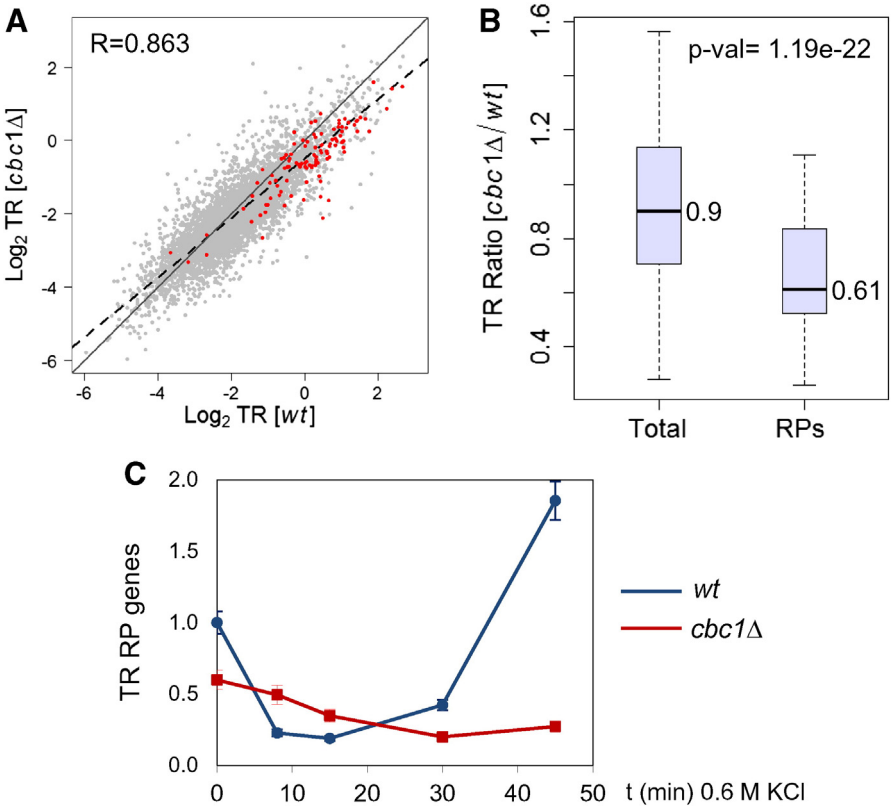


Fig. 7. Transcription of RP genes under non-stress and osmotic stress conditions is strongly affected in *cbc1Δ* mutant. (A) Plot of *cbc1Δ* TR against wild type TR for 5868 genes analyzed by GRO under non-stress conditions (time 0). The bisector is indicated as a solid line, and the trend line as a dark dotted line. Dots for RP genes are represented in red. (B) Box and whisker plot representations showing medians and quartiles of ratios between *cbc1Δ* TR and wild type TR of total yeast genes or only RP genes under non-stress conditions. The median of each data set is denoted and the whiskers show the data extremes, where outliers are discarded. Total data is compared with RP data and the p-value obtained from t-test is denoted. See the Materials and methods section for additional details about representations in (A) and (B). (C) TR median of all RP genes under 0.6 M KCl stress for the indicated times and non-stress conditions in wild type and *cbc1Δ*. Error bars represent SE of all RP genes analyzed. All data were taken from 3 independent GRO experiments as described in Fig. 1.

normal conditions, and observed that while *cbc1Δ*/wt ratio for global TR was 0.9, the ratio for RP genes was significantly lower (0.61; p-value 1.19e−22). This result indicates that the transcription of RP genes is importantly reduced compared to global TR under normal conditions in the absence of Cbc1 (Fig. 7B). The negative effect of *CBC1* deletion on TR of RP genes was also observed during the response to osmotic stress. Whereas in wt, the initial decrease in TR was followed by recovery after 45 min of stress, no recovery was observed in *cbc1Δ* at 45 min (Fig. 7C). Altogether, these results indicate that Cbc1 does not affect transcription of all genes equally, but affects some groups of genes, such RP genes, in which Cbc1 is required for their transcription both under normal conditions and during osmotic stress recovery.

3.8. Cbc1 mediates expression of RP genes through the accumulation of Rap1 and PIC at RP promoters

Our results have shown that Cbc1 mediates expression of osmotic stress-induced genes by facilitating the accumulation of the activator Hot1–Hog1 at promoters, which mediates the recruitment of PIC components and RNAPII (Figs. 1-6). We have also observed significantly lower TBP protein level in *cbc1Δ* mutant cells (Fig. 5), which may diminish the expression of many genes both under non-stress and stress conditions. We then investigated whether the low expression of RP genes was associated with deficient accumulation of the RP-specific activator Rap1 and TBP at RP promoters. Previously, it has been reported that Rap1 association with the promoter of some RP genes did not change during osmotic stress provoked by 0.4 M NaCl [82]; however, here we observed a drop in Rap1 association with *RPL30* and *RPL33B* promoters in wt cells after 10 min of treatment with 0.6 M KCl, which mostly recovered at 60 min of stress (Fig. 8). As shown in Fig. 8A, a reduced occupancy of both Rap1 and TBP was observed at *RPL30* and *RPL33B* promoters under non-stress conditions (time 0) and during the TR recovery during stress adaptation in *cbc1Δ* cells, indicating a role of Cbc1 in recruitment of the specific activator and PIC to RP promoters. We also analyzed Rap1 protein levels and observed no reduction in Rap1 protein in the absence of Cbc1; in fact, a slightly higher Rap1 protein expression was observed in *cbc1Δ* compared to wt and, therefore, the reduced binding of Rap1 to RP promoter in *cbc1Δ* was not a consequence of reduced Rap1 protein (Fig. 8B).

In all, our results suggest that, similarly to its role in osmotic stress-induced gene expression, the cap-binding protein Cbc1 is required to achieve high levels of transcription of RP genes through the accumulation of the specific activator Rap1 and TBP at RP promoters under normal conditions and during the recovery phase under osmotic stress. Interestingly, we again observed changes in the cellular protein level of a transcription activator, Rap1, when Cbc1 is absent.

3.9. Cbc1 modulates transcription of highly expressed genes and may be implicated in the response to various environmental stresses

Our work identifies a role for Cbc1 in transcription of osmostress-responsive genes which are highly induced during stress and also in transcription of the highly expressed RP genes. Additionally, studies by other authors describing Cbc1 in transcription involved genes with high transcription levels, such as *GAL* genes under induced conditions or the constitutively expressed *PMA1* and *ADH1* genes [31,38,42]. However, we have seen that transcription of all genes is not affected equally by a *CBC1* deletion (Figs. 1B and 7A and B). We therefore hypothesize that Cbc1 shows functional bias towards highly expressed genes. To check this hypothesis, we utilized our TR data under non-stress

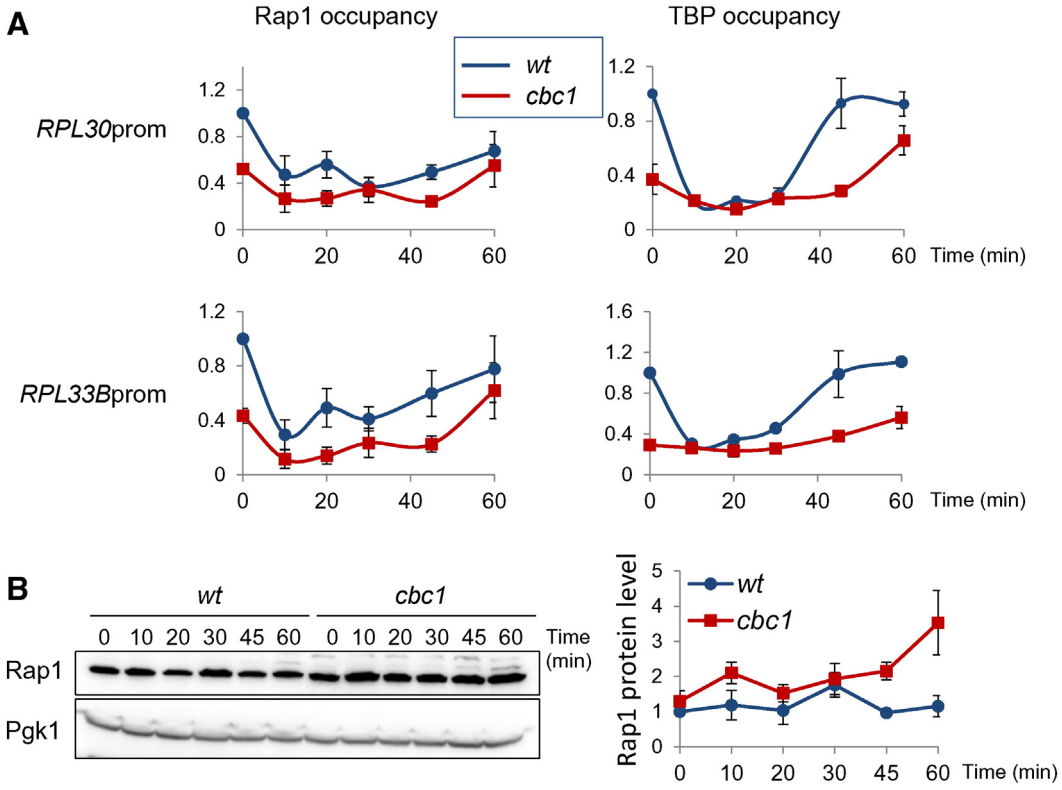


Fig. 8. Cbc1 regulates Rap1 recruitment and PIC formation at RP promoters and modulates Rap1 protein levels. (A) Graphical representations of ChIP analyses of Rap1 and TBP recruitment to the promoters of RP genes *RPL30* and *RPL33B* in wild type (blue line) and *cbc1Δ* (red line) during 0.6 M KCl treatment for 60 min. Immunoprecipitation of endogenous Rap1 was carried out using an anti-Rap1 (clone y-300) antibody. Immunoprecipitation of HA-TBP expressed in a centromeric plasmid (PA149) was carried out using an anti-HA antibody. Promoters were amplified by qPCR using specific primers, and signals were represented as a percentage of input, and then normalized against wild type non-stress of each experiment. Averages and SD of three independent experiments are represented graphically. (B) Left panel shows a representative western blot probed with anti-Rap1 and anti-Pgk1 antibodies to detect endogenous protein expressions of Rap1 and loading control Pgk1, respectively, before and after treatment with 0.6 M KCl at the indicated times. Right panel shows the average and standard deviations of band intensities from three independent experiments. Band intensities of Rap1 were normalized against the band intensity of Pgk1 for each sample, and all relative expressions were represented as a fold-change relative to wild type non-stress of each experiment.

conditions and compared the median of TR of the 10% highest and lowest expressing genes in wt and in *cbc1Δ* under normal conditions. We observed that although *cbc1Δ* showed a slight but significant increase in TR for lowest expressing genes, a marked decrease in TR was observed for highest expressing genes (Fig. 9). These results suggest that Cbc1, although it may regulate gene repression of low expressing genes, plays an essential role in maintaining a high TR for genes that are highly transcribed.

Due to the general role of Cbc1 as a transcriptional activator and its specific roles in the rapid and high transcription of osmostress genes in response to osmotic stress, it is expected that Cbc1, together with Cbc2, may have a similar role in the response to other environmental stresses, where rapid and strong up-regulation of genes for survival and adaptation are required. Here, we analyzed cellular growth of *cbc1Δ* and *cbc2Δ* mutants under different environmental stresses. Both *cbc1Δ* and *cbc2Δ* mutants show similar growth defects under $CaCl_2$ and cold stress (Fig. S3).

In summary, these results suggest that the cellular function of Cbc1 (together with Cbc2) is more general and may play a role in the regulation of gene expression in response to various environmental stresses.

4. Discussion

RNAPII transcription is a complex process involving diverse, yet distinct stages, such as initiation, elongation and termination, which respond to intracellular signaling in such a dynamic manner that the mRNA being synthesized is simultaneously modified and imprinted for its subsequent life [83,84]. Cross-talk between the factors involved in all these processes will determine the speed, intensity and length of transcription for each particular mRNA under each particular cellular condition [85,86]. In this context, interaction between factors of different processes and the existence of factors with multifunctional roles will be necessary to achieve this complex coordination. In this study, we define Cbc1 as a main multifunctional factor that coordinates several steps in transcription. Co-transcriptionally bound to the mRNA 5′ cap structure, recent studies have shown that Cbc1 is involved in PIC formation and recruitment of RNAPII to promoters during transcription initiation as well as in the efficient recruitment of the RNAPII kinases, Bur1 and Ctk1, to establish high levels of Ser2-phosphorylated CTD during transcription elongation of several genes [31,38,42,43]. Our work takes a step further to show that Cbc1 mediates the accumulation of gene-specific activators at promoters, which in turn recruit PIC components and new RNAPII molecules. We have also detected a physical interaction between the cap-binding protein and the central signaling factor of the osmotic stress response, the MAPK Hog1, which itself acts as a chromatin-binding activator by promoting the rapid recruitment of RNAPII upon osmotic shock [55,56]. Previously, Cbc1 has also been reported to function in splicing and transcription termination [4,5]. Therefore, our results, together with previously described data, demonstrate that Cbc1 connects RNA processing to all steps of transcription and also to signaling by accumulating signal-induced transcription activators at gene promoters.

4.1. Mechanism of Cbc1 regulation of transcription initiation

The reprogramming of transcription during the response to osmotic stress requires correct signaling and functional transcriptional machinery, but is also influenced by the proper activation of immediate

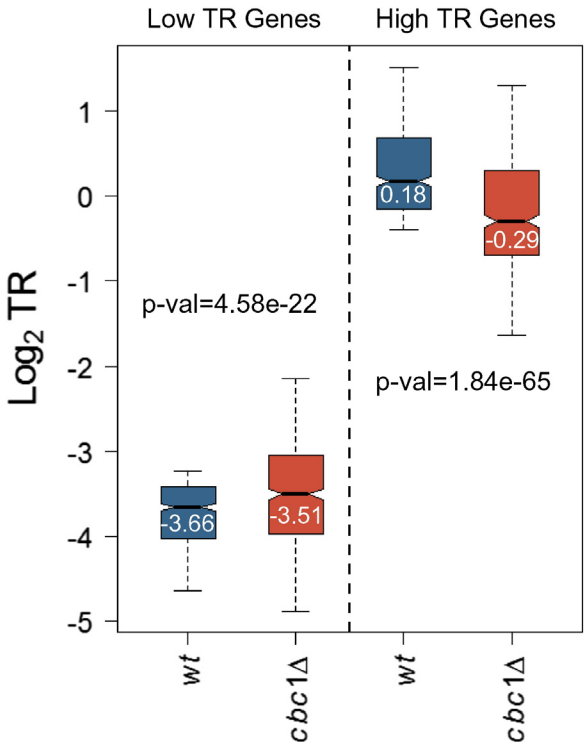


Fig. 9. Cbc1 show functional bias towards highly expressed genes. The box and whisker plots represent the median and quartiles of the data belonging to the genes with the lowest 10% TR (left) and the highest 10% TR (right), which were sorted from low to high with regards to the wild type TR levels under normal conditions. The numbers inside the boxes denotes the median value and the whiskers shows the data extremes (excluding the outliers which lie beyond 1 times the inter quartile range). The wild type TR (blue boxes) was compared to the TR of the same genes in the *cbc1Δ* mutant (red boxes) and the p-values from t-test are shown.

physiological responses [87]. However, our results favor a model where a direct participation of Cbc1 in transcription initiation by promoting the accumulation of activators at gene promoters is required to achieve high levels of gene transcription. The evidence we provide for this model stems from results obtained under both stress and non-stress conditions. First, we observe that Cbc1 is required for the transcriptional response to osmotic stress, as a deletion in *CBC1* causes a global alteration in TR profiles. More specifically, TR and RA induction of osmostress-induced genes are delayed and/or reduced in *cbc1Δ*, which is coupled with a reduction in TR and RA recoveries of the genes repressed in response to osmotic stress (Figs. 1-3). Second, our analyses have revealed that Cbc1 is required for the recruitment of the Hot1–Hog1 activator complex to Hot1-dependent osmostress promoters, and we have detected a physical interaction between Cbc1 and Hog1 (Figs. 5 and 6). Third, Cbc1 mediates the recruitment of RNAPII and formation of PIC at the promoters during osmotic stress, which may be a consequence of the role of Cbc1 in the recruitment of the activator (Figs. 3 and 5). Fourth, no delay in Hot1 and Hog1 phosphorylation by the HOG signaling pathway has been observed in the *cbc1Δ* mutant; therefore, the delayed accumulation of the Hot1–Hog1 complex at promoters is not a consequence of defective signaling (Fig. 3). Fifth, under normal growth conditions, Cbc1 is required for the high expression of RP genes and for the accumulation of their gene-specific activator Rap1 and PIC at the promoters (Figs. 7 and 8). And finally, during osmotic stress, Cbc1 appears to play a minor role in the rapid downregulation of RP expression; however, it is required for the recovery of transcription mediated by Rap1 and TBP binding at RP promoters (Fig. 8).

Cbc1 binds to the cap through its dynamic interaction with the cap-binding CBC subunit Cbc2, and this interaction is essential for the binding of CBC to the cap [2,74,88]. Although a cap-independent Cbc1 function in transcription cannot be dismissed, previous studies and our data strongly suggest that the function of Cbc1 in transcription initiation is executed through its binding to the capped mRNA. First, we have shown that a *cbc2* mutant displays similar stress-sensitive phenotypes and gene expression defects as *cbc1Δ* (Figs. 4 and S3), and also previous results show that defects in the induction of *GAL* genes are similar for *cbc1Δ* and *cbc2Δ* mutants [31]; second, in *GAL* genes, regulation of PIC formation by CBC seems to occur after mRNA capping, since depletion of the capping enzymes, Cet1 and Ceg1, also causes defective TBP and RNAPII recruitment [31]. Third, the deletion of Cbc1 has been previously shown to significantly reduce the stability of Cbc2, whereas the stability of Cbc1 is not dependent on the presence of Cbc2, indicating that in a *cbc2Δ* mutant, there is sufficient Cbc1 protein but is unable to bind the 5′ mRNA cap [1,2,6]. Therefore, it is more likely that the molecular functions on transcription of the two subunits of CBC are synergetic, inter-dependent and linked to their abilities to bind the cap.

The Cbc1/Cbc2 complex binds the mRNA 5′ cap during transcription elongation when the mRNA is approximately 25 nucleotides in length [4,89], indicating that mRNA-bound CBC does not play a role in the pioneer round of transcription. However, CBC may regulate subsequent rounds of transcription once bound to the mRNA cap by facilitating the stable accumulation of activators at the promoter [90]. Indeed, this is also supported by the observation that the deletion of Cbc1 does not completely abolish transcription of Cbc1-dependent genes, but instead delays and reduces induction of these genes. In this respect, a similar mechanism has been recently demonstrated in which nascent RNA enhances the occupancy of certain TFs at gene regulatory elements, which would contribute to the stability of gene expression programs [91]. For Cbc1, the accumulation of sequence-specific activators at promoters may work through the stabilization of activator/DNA interaction or the interaction between the activator and other components of the PIC. Additionally, we have documented a reduced binding of TBP to osmotic-induced promoters and RP promoters under stress and non-stress conditions in the absence of Cbc1 (Figs. 5 and 8), and it has been described that stable binding of TBP at promoters facilitates the reinitiation of transcription [92]. Therefore, a lower level of TBP at promoters caused by the absence of Cbc1 would explain the inability to reach high transcription rates in the *cbc1Δ* mutant. Our data suggest that reduced TBP binding is caused by reduced accumulation of activators at promoters; however, it is entirely possible that Cbc1 may regulate the binding of both activators and TBP in mechanistically distinct manners. Previous work using several galactose-induced *GAL* genes showed that CBC interacts and recruits Mot1, which regulates TBP binding to promoters of *GAL* genes [31,76], and it has also been shown previously that Mot1 binds stress-regulated promoters [37]. However, the up-regulation kinetics of osmostress mRNAs in conditions of Mot1 depletion do not show delayed induction, whereas under the same conditions depletion of Cbc1 provokes clear altered kinetics of osmo-mRNAs (Fig. S2). Therefore it is likely that the transcriptional contribution of Mot1 to the expression of specific genes is not equal, in that it is required for *GAL* and not for osmo-responsive genes.

Interestingly, our results hint at a major role of Cbc1 in the regulation of transcription for some specific groups of genes. We show Cbc1 to mediate transcription of genes highly induced by osmotic stress and also highly transcribed RP genes under normal conditions and during the recovery phase during osmotic stress. Previous studies show that Cbc1 regulates the transcription of induced *GAL* genes and two constitutive genes, *PMA1* and *ADH1* [31,38,42]; all of these genes showing high transcription levels under the investigated conditions. Indeed, our results under unstressed conditions, indicate that transcription of the highest transcribed genes is significantly reduced in *cbc1Δ* mutant, and conversely, transcription of the lowest transcribed genes is even slightly augmented in *cbc1Δ* relative to wt (Fig. 9). Previous studies have shown that Cbc1/Cbp80 deletion leads to a altered expression of a subset of yeast genes (around 6%) [93], and the deregulation of around

400 genes in mammalian cells [9]. Therefore, deletion of the cap-binding protein affects a large number of genes, but there is no systematic down-regulation of expression of all genes. We believe that most of the specificity of Cbc1 regulation of transcription depends on the transcription rate of the gene and/or the velocity of its induction kinetics. For highly transcribed genes, Cbc1 could facilitate subsequent rounds of transcription through the stable interaction of gene-specific activators with promoters and this may not be restricted to only one or two specific activators. In fact, although we report here that Cbc1 recruits the Hot1–Hog1 activator complex to a subset of osmostress-induced genes, we also show that Cbc1-dependent transcription under osmotic stress is not restricted to Hog1-dependent genes or one particular osmostress activator, suggesting that Cbc1 may function to positively recruit other gene-specific activators to other up-regulated or highly expressed genes. This is the case for establishing high transcription levels in RP genes, which is mediated by Cbc1 through the accumulation of Rap1.

Taken together, we propose a model in which highly transcribed genes, containing high density of RNAPII, recruit more CBC to bind to the mRNA 5′ cap. Once bound, CBC aids subsequent rounds of transcription initiation and formation of PIC through the accumulation at promoters of gene-specific activators in a positive feedback loop. Such a loop would cause an exponential increase in the TR of activated genes, explaining the enhanced defect of highly transcribed genes in *cbc1Δ* mutant. Given the number and variety of genes whose transcription is affected in *cbc1Δ*, and the different specific activators involved, the role of Cbc1 in activator accumulation at promoters could be mediated by other means than physical interactions with all the activators. As explained above, Cbc1 could exert a positive effect in the binding of TBP. Alternative possibilities are that Cbc1 could stabilize mediator components or other general transcription factors (GTF) at promoters or mediate changes in the chromatin at the promoter regions. In the context of the latter possibility, recent work has determined that a differential and specific nucleosomal architecture is established at the promoters of highly transcribed genes [94], which could further hint at the possible role of Cbc1 in chromatin remodeling. Further experiments should be performed to fully understand how Cbc1 mediates the accumulation of different specific activators at promoters.

4.2. Cbc1 impacts the regulation of activator protein levels

We have observed that deletion of *CBC1* appears to change the expression of the Cbc1-dependent chromatin binding activators Hot1, Rap1 and TBP (Figs. 5 and 8). In the case of Hot1 and Rap1, a higher cellular protein level is observed in a *cbc1Δ* mutant; therefore the reduced promoter binding observed is not caused by lower protein levels. In the case of TBP, the significant lower cellular protein level observed in *cbc1Δ* could explain the reduced TBP and RNAPII binding. Alternatively, it is possible that the decreased promoter accumulation of these transcription activators in absence of Cbc1 in turn causes a change in their protein stabilities [95], as such that decreased promoter association results in increased Hot1 and Rap1 stability. Similarly, TBP protein may be destabilized due to a decrease in its chromatin association as a consequence of reduced Hot1–Hog1 recruitment in *cbc1Δ*. Our hypothesis is supported by previous studies showing that the ubiquitin protease Ubp3, which reverses the ubiquitination of TBP during transcriptional activation [96], is recruited by Hog1 to osmostress-inducible promoters where it modulates transcription, suggesting that Ubp3 could protect promoter-bound TBP from degradation [97]. Moreover, a connection between recruitment of gene-specific activators to chromatin and enhanced degradation has been established by other studies [98,99], and attenuation of the stress response has been connected to nuclear protein modification and degradation of such activators as Msn2 [100,101]. Furthermore, our results also reveal a delay in HOG signaling down-regulation in *cbc1Δ*, as Hot1 and Hog1 remain phosphorylated for longer following osmotic stress exposure, which correlates with decreased promoter binding of these factors (Fig. 5), suggesting that dephosphorylation occurs on chromatin-bound proteins.

4.3. Biological relevance of Cbc1 function in gene expression under normal conditions and during the stress response

All results presented here indicate that in the absence of Cbc1, there are important but transcription rate-dependent changes in mRNA synthesis which can cause growth defects under both normal and osmotic stress conditions. Indeed, transcription of ribosomal protein genes is reduced by around 40% in *cbc1Δ* mutant relative to wt (Fig. 7) and lower RP expression has been shown to correlate with lower growth rate [102]. Accordingly, we have observed a longer duplication time (2.1 h) in rich media under normal conditions in *cbc1Δ* mutant compared to wt (1.5 h). We have also shown that *cbc1Δ* mutants show growth defects and lower viability under osmotic stress [29], however, the function of Cbc1 upon osmostress goes beyond its role in transcription, since we have previously documented that Cbc1 associates with polyribosomes in active translation and, moreover, the absence of Cbc1 reduces the percentage of osmostress mRNAs engaging in active translation [29]. The effect of Cbc1 in expression of osmo-mRNAs during osmotic stress, both transcriptional and translational, could explain the low viability of the *cbc1* mutant in hyperosmotic media. Additionally, in *Arabidopsis*, the cap-binding proteins Cbp80 and Cbp20 modulate the response to osmotic stress through the regulation of splicing, and hence expression, of several genes involved in sugar and proline metabolism [59]. Yeast Cbc1 also has important roles in splicing and specifically in the processing of the introns of RP genes [103], whose pre-mRNAs are also highly regulated in response to osmotic stress [50]. All this evidence suggests that Cbc1 has multifunctional roles during osmotic stress, and acts as a key factor coordinating different levels of gene expression. The cellular response to other environmental stresses also requires high and timely transcription of protective genes and down-regulation and later recovery of housekeeping genes, such as RP genes [104]. Our observation that cellular growth under various stresses requires Cbc1 and Cbc2 (Fig. S3), suggests that the multifunctional roles in gene expression of the mRNA cap-binding proteins are necessary to attain an adequate response to stress.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgments

The authors are grateful to the members of the laboratories in Valencia and Göteborg for the discussion and support, and also to Fany Carrasco for the technical assistance. We thank Gustav Ammerer and Aurora Zuzuarregui for the M-track plasmids and advice, and Kevin Struhl for the TBP plasmid. This work was supported by the Spanish Ministerio de Ciencia e Innovación [BFU2008-02114 to P.A., BFU2013-48643-C3-3-P to J.E.P.-O. and P. A.]; European Union funds (FEDER); the Regional Valencian Government [GVACOMP2011-105 to P.A., PROMETEOII 2015/006 and GVACOMP/2012/001 to J.E.P.-O.]; Universitat de València [UV-INV-AE13-139034 to P.A.]; the Swedish Research Council [2010-4645 to P.S.] and by the Carl Trygger Foundation [KF13:8 to P.S.]. T. L. was recipient of a PROMETEO predoctoral contract of the Generalitat Valenciana (PROMETEO 2011/088) and P.A. was supported by the Guest Researcher program 2011-12 of the University of Gothenburg.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbagrm.2016.01.002.

References

[1] E. Izaurralde, J. Lewis, C. McGuigan, M. Jankowska, E. Darzynkiewicz, et al., A nuclear cap binding protein complex involved in pre-mRNA splicing, Cell 78 (1994) 657–668.
[2] E. Izaurralde, J. Lewis, C. Gamberi, A. Jarmolowski, C. McGuigan, et al., A cap-binding protein complex mediating U snRNA export, Nature 376 (1995) 709–712.
[3] N. Sonenberg, A.G. Hinnebusch, Regulation of translation initiation in eukaryotes: mechanisms and biological targets, Cell 136 (2009) 731–745.
[4] T. Gonatopoulos-Pournatzis, V.H. Cowling, Cap-binding complex (CBC), Biochem. J. 457 (2014) 231–242.
[5] I. Topisirovic, Y.V. Svitkin, N. Sonenberg, A.J. Shatkin, Cap and cap-binding proteins in the control of gene expression, Wiley interdisciplinary reviews RNA, 2 2011, pp. 277–298.
[6] C.M. Wong, H. Qiu, C. Hu, J. Dong, A.G. Hinnebusch, Yeast cap binding complex impedes recruitment of cleavage factor IA to weak termination sites, Mol. Cell. Biol. 27 (2007) 6520–6531.
[7] P. Fortes, J. Kufel, M. Fornerod, M. Polycarpou-Schwarz, D. Lafontaine, et al., Genetic and physical interactions involving the yeast nuclear cap-binding complex, Mol. Cell. Biol. 19 (1999) 6543–6553.
[8] V. Hugouvieux, J.M. Kwak, J.I. Schroeder, An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in *Arabidopsis*, Cell 106 (2001) 477–487.
[9] T. Narita, T.M. Yung, J. Yamamoto, Y. Tsuboi, H. Tanabe, et al., NELF interacts with CBC and participates in 3′ end processing of replication-dependent histone mRNAs, Mol. Cell 26 (2007) 349–365.
[10] J.D. Lewis, E. Izaurralde, A. Jarmolowski, C. McGuigan, I.W. Mattaj, A nuclear cap-binding complex facilitates association of U1 snRNP with the cap-proximal 5′ splice site, Genes Dev. 10 (1996) 1683–1698.
[11] J.D. Lewis, D. Gorlich, I.W. Mattaj, A yeast cap binding protein complex (yCBC) acts at an early step in pre-mRNA splicing, Nucleic Acids Res. 24 (1996) 3332–3336.
[12] H.V. Colot, F. Stutz, M. Rosbash, The yeast splicing factor Mud13p is a commitment complex component and corresponds to CBP20, the small subunit of the nuclear cap-binding complex, Genes Dev. 10 (1996) 1699–1708.
[13] J. Gornemann, K.M. Kotovic, K. Hujer, K.M. Neugebauer, Cotranscriptional spliceosome assembly occurs in a stepwise fashion and requires the cap binding complex, Mol. Cell 19 (2005) 53–63.
[14] S.M. Flaherty, P. Fortes, E. Izaurralde, I.W. Mattaj, G.M. Gilmartin, Participation of the nuclear cap binding complex in pre-mRNA 3′ processing, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 11893–11898.
[15] B. Das, Z. Guo, P. Russo, P. Chartrand, F. Sherman, The role of nuclear cap binding protein Cbc1p of yeast in mRNA termination and degradation, Mol. Cell. Biol. 20 (2000) 2827–2838.
[16] N. Visa, E. Izaurralde, J. Ferreira, B. Daneholt, I.W. Mattaj, A nuclear cap-binding complex binds Balbiani ring pre-mRNA cotranscriptionally and accompanies the ribonucleoprotein particle during nuclear export, J. Cell Biol. 133 (1996) 5–14.
[17] H. Cheng, K. Dufu, C.S. Lee, J.L. Hsu, A. Dias, et al., Human mRNA export machinery recruited to the 5′ end of mRNA, Cell 127 (2006) 1389–1400.
[18] E.C. Shen, T. Stage-Zimmermann, P. Chui, P.A. Silver, The yeast mRNA-binding protein Npl3p interacts with the cap-binding complex, J. Biol. Chem. 275 (2000) 23718–23724.
[19] E.P. Lei, H. Krebber, P.A. Silver, Messenger RNAs are recruited for nuclear export during transcription, Genes Dev. 15 (2001) 1771–1782.
[20] E. Grudzien, M. Kalek, J. Jemielity, E. Darzynkiewicz, R.E. Rhoads, Differential inhibition of mRNA degradation pathways by novel cap analogs, J. Biol. Chem. 281 (2006) 1857–1867.
[21] N.A. Balatsos, P. Nilsson, C. Mazza, S. Cusack, A. Virtanen, Inhibition of mRNA deadenylation by the nuclear cap binding complex (CBC), J. Biol. Chem. 281 (2006) 4517–4522.
[22] L. Kuai, B. Das, F. Sherman, A nuclear degradation pathway controls the abundance of normal mRNAs in *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 13962–13967.
[23] B. Das, J.S. Butler, F. Sherman, Degradation of normal mRNA in the nucleus of *Saccharomyces cerevisiae*, Mol. Cell. Biol. 23 (2003) 5502–5515.
[24] P. Fortes, T. Inada, T. Preiss, M.W. Hentze, I.W. Mattaj, et al., The yeast nuclear cap binding complex can interact with translation factor eIF4G and mediate translation initiation, Mol. Cell 6 (2000) 191–196.
[25] S.Y. Chiu, F. Lejeune, A.C. Ranganathan, L.E. Maquat, The pioneer translation initiation complex is functionally distinct from but structurally overlaps with the steady-state translation initiation complex, Genes Dev. 18 (2004) 745–754.
[26] H. Sato, L.E. Maquat, Remodeling of the pioneer translation initiation complex involves translation and the karyopherin importin beta, Genes Dev. 23 (2009) 2537–2550.
[27] A.G. Hinnebusch, J.R. Lorsch, The mechanism of eukaryotic translation initiation: new insights and challenges, Cold Spring Harb. Perspect. Biol. 4 (2012).
[28] A. Sharma, A. Yilmaz, K. Marsh, A. Cochrane, K. Boris-Lawrie, Thriving under stress: selective translation of HIV-1 structural protein mRNA during Vpr-mediated impairment of eIF4E translation activity, PLoS Pathog. 8 (2012), e1002612.
[29] E. Garre, L. Romero-Santacreu, N. De Clercq, N. Blasco-Angulo, P. Sunnerhagen, et al., Yeast mRNA cap-binding protein Cbc1/Sto1 is necessary for the rapid reprogramming of translation after hyperosmotic shock, Mol. Biol. Cell 23 (2012) 137–150.
[30] N. Oh, K.M. Kim, J. Choe, Y.K. Kim, Pioneer round of translation mediated by nuclear cap-binding proteins CBP80/20 occurs during prolonged hypoxia, FEBS Lett. 581 (2007) 5158–5164.
[31] S. Lahudkar, A. Shukla, P. Bajwa, G. Durairaj, N. Stanojevic, et al., The mRNA cap-binding complex stimulates the formation of pre-initiation complex at the promoter via its interaction with Mot1p in vivo, Nucleic Acids Res. 39 (2011) 2188–2209.
[32] A. Dasgupta, R.P. Darst, K.J. Martin, C.A. Afshari, D.T. Auble, Mot1 activates and represses transcription by direct, ATPase-dependent mechanisms, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 2666–2671.
[33] P. Wollmann, S. Cui, R. Viswanathan, O. Berninghausen, M.N. Wells, et al., Structure and mechanism of the Swi2/Snf2 remodeller Mot1 in complex with its substrate TBP, Nature 475 (2011) 403–407.
[34] R.O. Sprouse, T.S. Karpova, F. Mueller, A. Dasgupta, J.G. McNally, et al., Regulation of TATA-binding protein dynamics in living yeast cells, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 13304–13308.
[35] P. de Graaf, F. Mousson, B. Geverts, E. Scheer, L. Tora, et al., Chromatin interaction of TATA-binding protein is dynamically regulated in human cells, J. Cell Sci. 123 (2010) 2663–2671.
[36] F.J. van Werven, H. van Bakel, H.A. van Teeffelen, A.F. Altelaar, M.G. Koerkamp, et al., Cooperative action of NC2 and Mot1p to regulate TATA-binding protein function across the genome, Genes Dev. 22 (2008) 2359–2369.
[37] J.V. Geisberg, K. Struhl, Cellular stress alters the transcriptional properties of promoter-bound Mot1–TBP complexes, Mol. Cell 14 (2004) 479–489.
[38] M. Lidschreiber, K. Leike, P. Cramer, Cap completion and C-terminal repeat domain kinase recruitment underlie the initiation–elongation transition of RNA polymerase II, Mol. Cell. Biol. 33 (2013) 3805–3816.
[39] S. Lahudkar, G. Durairaj, B. Uprety, S.R. Bhaumik, A novel role for Cet1p mRNA 5′-triphosphatase in promoter proximal accumulation of RNA polymerase II in *Saccharomyces cerevisiase*, Genetics 196 (2014) 161–176.
[40] P.A. Schroder, M.J. Moore, Association of ribosomal proteins with nascent transcripts in *S. cerevisiae*, RNA 11 (2005) 1521–1529.
[41] S.C. Schroeder, D.A. Zorio, B. Schwer, S. Shuman, D. Bentley, A function of yeast mRNA cap methyltransferase, Abd1, in transcription by RNA polymerase II, Mol. Cell 13 (2004) 377–387.
[42] M.A. Hossain, C. Chung, S.K. Pradhan, T.L. Johnson, The yeast cap binding complex modulates transcription factor recruitment and establishes proper histone H3K36 trimethylation during active transcription, Mol. Cell. Biol. 33 (2013) 785–799.
[43] T. Lenasi, B.M. Peterlin, M. Barboric, Cap-binding protein complex links pre-mRNA capping to transcription elongation and alternative splicing through positive transcription elongation factor b (P-TEFb), J. Biol. Chem. 286 (2011) 22758–22768.
[44] S. Hohmann, M. Krantz, B. Nordlander, Yeast osmoregulation, Methods Enzymol. 428 (2007) 29–45.
[45] S. Hohmann, Osmotic stress signaling and osmoadaptation in yeasts, Microbiol. Mol. Biol. Rev. 66 (2002) 300–372.
[46] F. Posas, J.R. Chambers, J.A. Heyman, J.P. Hoeffler, E. de Nadal, et al., The transcriptional response of yeast to saline stress, J. Biol. Chem. 275 (2000) 17249–17255.
[47] C. Molin, A. Jauhiainen, J. Warringer, O. Nerman, P. Sunnerhagen, mRNA stability changes precede changes in steady-state mRNA amounts during hyperosmotic stress, RNA 15 (2009) 600–614.
[48] L. Romero-Santacreu, J. Moreno, J.E. Perez-Ortin, P. Alepuz, Specific and global regulation of mRNA stability during osmotic stress in *Saccharomyces cerevisiae*, RNA 15 (2009) 1110–1120.
[49] J. Warringer, M. Hult, S. Regot, F. Posas, P. Sunnerhagen, The HOG pathway dictates the short-term translational response after hyperosmotic shock, Mol. Biol. Cell 21 (2010) 3080–3092.
[50] E. Garre, L. Romero-Santacreu, M. Barneo-Munoz, A. Miguel, J.E. Perez-Ortin, et al., Nonsense-mediated mRNA decay controls the changes in yeast ribosomal protein pre-mRNAs levels upon osmotic stress, PLoS One 8 (2013), e61240.
[51] E. de Nadal, G. Ammerer, F. Posas, Controlling gene expression in response to stress, Nat. Rev. Genet. 12 (2011) 833–845.
[52] H. Saito, F. Posas, Response to hyperosmotic stress, Genetics 192 (2012) 289–318.
[53] F. Martinez-Montanes, A. Pascual-Ahuir, M. Proft, Toward a genomic view of the gene expression program regulated by osmostress in yeast, Omics 14 (2010) 619–627.
[54] E. de Nadal, P.M. Alepuz, F. Posas, Dealing with osmostress through MAP kinase activation, EMBO Rep. 3 (2002) 735–740.
[55] P.M. Alepuz, A. Jovanovic, V. Reiser, G. Ammerer, Stress-induced map kinase Hog1 is part of transcription activation complexes, Mol. Cell 7 (2001) 767–777.
[56] P.M. Alepuz, E. de Nadal, M. Zapater, G. Ammerer, F. Posas, Osmostress-induced transcription by Hot1 depends on a Hog1-mediated recruitment of the RNA Pol II, EMBO J. 22 (2003) 2433–2442.
[57] K.E. Cook, E.K. O'Shea, Hog1 Controls Global Reallocation of RNA Pol II Upon Osmotic Shock in *Saccharomyces cerevisiae*, G3 22012 1129–1136.
[58] M. Nadal-Ribelles, N. Conde, O. Flores, J. Gonzalez-Vallinas, E. Eyras, et al., Hog1 bypasses stress-mediated down-regulation of transcription by RNA polymerase II redistribution and chromatin remodeling, Genome Biol. 13 (2012) R106.
[59] X. Kong, L. Ma, L. Yang, Q. Chen, N. Xiang, et al., Quantitative proteomics analysis reveals that the nuclear cap-binding complex proteins *Arabidopsis* CBP20 and CBP80 modulate the salt stress response, J. Proteome Res. 13 (2014) 2495–2510.
[60] J. Garcia-Martinez, A. Aranda, J.E. Perez-Ortin, Genomic run-on evaluates transcription rates for all yeast genes and identifies gene regulatory mechanisms, Mol. Cell 15 (2004) 303–313.
[61] Team RC, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, 2013.
[62] E. Eden, R. Navon, I. Steinfeld, D. Lipson, Z. Yakhini, GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists, BMC Bioinforma. 10 (2009) 48.
[63] F. Supek, M. Bosnjak, N. Skunca, T. Smuc, REVIGO summarizes and visualizes long lists of gene ontology terms, PLoS One 6 (2011) e21800.

[64] M.C. Teixeira, P.T. Monteiro, J.F. Guerreiro, J.P. Goncalves, N.P. Mira, et al., The YEASTRACT database: an upgraded information system for the analysis of gene and genomic transcription regulation in *Saccharomyces cerevisiae*, Nucleic Acids Res. 42 (2014) D161–D166.
[65] J.M. Cherry, E.L. Hong, C. Amundsen, R. Balakrishnan, G. Binkley, et al., *Saccharomyces* Genome Database: the genomics resource of budding yeast, Nucleic Acids Res. 40 (2012) D700–D705.
[66] I. Medina, J. Carbonell, L. Pulido, S.C. Madeira, S. Goetz, et al., Babelomics: an integrative platform for the analysis of transcriptomics, proteomics and genomic data with advanced functional profiling, Nucleic Acids Res. 38 (2010) W210–W213.
[67] T. Li, B. Belda-Palazon, A. Ferrando, P. Alepuz, Fertility and polarized cell growth depends on eIF5A for translation of polyproline-rich formins in *Saccharomyces cerevisiae*, Genetics 197 (2014) 1191–1200.
[68] A. Miguel, F. Monton, T. Li, F. Gomez-Herreros, S. Chavez, et al., External conditions inversely change the RNA polymerase II elongation rate and density in yeast, Biochim. Biophys. Acta 1829 (2013) 1248–1255.
[69] A. Zuzuarregui, T. Kupka, B. Bhatt, I. Dohnal, I. Mudrak, et al., M-Track: detecting short-lived protein–protein interactions in vivo, Nat. Methods 9 (2012) 594–596.
[70] A. Zuzuarregui, T. Li, C. Friedmann, G. Ammerer, P. Alepuz, Msb2 is a Ste11 membrane concentrator required for full activation of the HOG pathway, Biochim. Biophys. Acta 1849 (2015) 722–730.
[71] M. Gomar-Alba, P. Alepuz, M. de Olmo, Dissection of the elements of osmotic stress response transcription factor Hot1 involved in the interaction with MAPK Hog1 and in the activation of transcription, Biochim. Biophys. Acta 1829 (2013) 1111–1125.
[72] M. Rep, V. Reiser, U. Gartner, J.M. Thevelein, S. Hohmann, et al., Osmotic stress-induced gene expression in *Saccharomyces cerevisiae* requires Msn1p and the novel nuclear factor Hot1p, Mol. Cell. Biol. 19 (1999) 5474–5485.
[73] A. Garay-Arroyo, A.A. Covarrubias, Three genes whose expression is induced by stress in *Saccharomyces cerevisiae*, Yeast 15 (1999) 879–892.
[74] G. Calero, K.F. Wilson, T. Ly, J.L. Rios-Steiner, J.C. Clardy, et al., Structural basis of m7GpppG binding to the nuclear cap-binding protein complex, Nat. Struct. Biol. 9 (2002) 912–917.
[75] R. Worch, M. Jankowska-Anyszka, A. Niedzwiecka, J. Stepinski, C. Mazza, et al., Diverse role of three tyrosines in binding of the RNA 5′ cap to the human nuclear cap binding complex, J. Mol. Biol. 385 (2009) 618–627.
[76] G.E. Zentner, S. Henikoff, Mot1 redistributes TBP from TATA-containing to TATA-less promoters, Mol. Cell. Biol. 33 (2013) 4996–5004.
[77] I. Topalidou, M. Papamichos-Chronakis, G. Thireos, D. Tzamarias, Spt3 and Mot1 cooperate in nucleosome remodeling independently of TBP recruitment, EMBO J. 23 (2004) 1943–1948.
[78] T.W. Sikorski, S. Buratowski, The basal initiation machinery: beyond the general transcription factors, Curr. Opin. Cell Biol. 21 (2009) 344–351.
[79] K.P. Hopfner, C.B. Gerhold, K. Lakomek, P. Wollmann, Swi2/Snf2 remodelers: hybrid views on hybrid molecular machines, Curr. Opin. Struct. Biol. 22 (2012) 225–233.
[80] M. Teige, E. Scheikl, V. Reiser, H. Ruis, G. Ammerer, Rck2, a member of the calmodulin-protein kinase family, links protein synthesis to high osmolarity MAP kinase signaling in budding yeast, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 5625–5630.
[81] J.L. Brewster, T. de Valoir, N.D. Dwyer, E. Winter, M.C. Gustin, An osmosensing signal transduction pathway in yeast, Science 259 (1993) 1760–1763.
[82] J.T. Wade, D.B. Hall, K. Struhl, The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes, Nature 432 (2004) 1054–1058.
[83] B.J. Venters, B.F. Pugh, How eukaryotic genes are transcribed, Crit. Rev. Biochem. Mol. Biol. 44 (2009) 117–141.
[84] G. Haimovich, M. Choder, R.H. Singer, T. Trcek, The fate of the messenger is pre-determined: a new model for regulation of gene expression, Biochim. Biophys. Acta 1829 (2013) 643–653.
[85] M.J. Moore, N.J. Proudfoot, Pre-mRNA processing reaches back to transcription and ahead to translation, Cell 136 (2009) 688–700.
[86] J.E. Perez-Ortin, P. Alepuz, S. Chavez, M. Choder, Eukaryotic mRNA decay: methodologies, pathways, and links to other stages of gene expression, J. Mol. Biol. 425 (2013) 3750–3775.
[87] E. Vanacloig-Pedros, C. Bets-Plasencia, A. Pascual-Ahuir, M. Proft, Coordinated gene regulation in the initial phase of salt stress adaptation, J. Biol. Chem. 290 (2015) 10163–10175.
[88] C. Mazza, M. Ohno, A. Segref, I.W. Mattaj, S. Cusack, Crystal structure of the human nuclear cap binding complex, Mol. Cell 8 (2001) 383–396.
[89] J. Shandilya, S.G. Roberts, The transcription cycle in eukaryotes: from productive initiation to RNA polymerase II recycling, Biochim. Biophys. Acta 1819 (2012) 391–400.
[90] Y. Wang, F. Liu, W. Wang, Dynamic mechanism for the transcription apparatus orchestrating reliable responses to activators, Sci. Rep. 2 (2012) 422.
[91] A.A. Sigova, B.J. Abraham, X. Ji, B. Molinie, N.M. Hannett, et al., Transcription factor trapping by RNA in gene regulatory elements, Science 350 (2015) 978–981.
[92] K. Poorey, R.O. Sprouse, M.N. Wells, R. Viswanathan, S. Bekiranov, et al., RNA synthesis precision is regulated by preinitiation complex turnover, Genome Res. 20 (2010) 1679–1688.
[93] M.A. Hossain, J.M. Claggett, T. Nguyen, T.L. Johnson, The cap binding complex influences H2B ubiquitination by facilitating splicing of the SUS1 pre-mRNA, RNA 15 (2009) 1515–1527.
[94] S. Kubik, M.J. Bruzzone, P. Jacquet, J.L. Falcone, J. Rougemont, et al., Nucleosome stability distinguishes two different promoter types at all protein-coding genes in yeast, Mol. Cell 60 (2015) 422–434.
[95] B.F. Pugh, Control of gene expression through regulation of the TATA-binding protein, Gene 255 (2000) 1–14.
[96] B.S. Chew, W.L. Siew, B. Xiao, N. Lehming, Transcriptional activation requires protection of the TATA-binding protein Tbp1 by the ubiquitin-specific protease Ubp3, Biochem. J. 431 (2010) 391–399.
[97] C. Sole, M. Nadal-Ribelles, C. Kraft, M. Peter, F. Posas, et al., Control of Ubp3 ubiquitin protease activity by the Hog1 SAPK modulates transcription upon osmostress, EMBO J. 30 (2011) 3274–3284.
[98] E. Molinari, M. Gilman, S. Natesan, Proteasome-mediated degradation of transcriptional activators correlates with activation domain potency in vivo, EMBO J. 18 (1999) 6439–6447.
[99] E. Rosonina, S.M. Duncan, J.L. Manley, Sumoylation of transcription factor Gcn4 facilitates its Srb10-mediated clearance from promoters in yeast, Genes Dev. 26 (2012) 350–355.
[100] S. Bose, J.A. Dutko, R.S. Zitomer, Genetic factors that regulate the attenuation of the general stress response of yeast, Genetics 169 (2005) 1215–1226.
[101] Y. Chi, M.J. Huddleston, X. Zhang, R.A. Young, R.S. Annan, et al., Negative regulation of Gcn4 and Msn2 transcription factors by Srb10 cyclin-dependent kinase, Genes Dev. 15 (2001) 1078–1092.
[102] E. O'Duibhir, P. Lijnzaad, J.J. Benschop, T.L. Lenstra, D. van Leenen, et al., Cell cycle population effects in perturbation studies, Mol. Syst. Biol. 10 (2014) 732.
[103] M. Bragulat, M. Meyer, S. Macias, M. Camats, M. Labrador, et al., RPL30 regulation of splicing reveals distinct roles for Cbp80 in U1 and U2 snRNP cotranscriptional recruitment, RNA 16 (2010) 2033–2041.
[104] D. Canadell, J. Garcia-Martinez, P. Alepuz, J.E. Perez-Ortin, J. Arino, Impact of high pH stress on yeast gene expression: a comprehensive analysis of mRNA turnover during stress responses, Biochim. Biophys. Acta 1849 (2015) 653–664.
[105] E. Bilsland-Marchesan, J. Arino, H. Saito, P. Sunnerhagen, F. Posas, Rck2 kinase is a substrate for the osmotic stress-activated mitogen-activated protein kinase Hog1, Mol. Cell. Biol. 20 (2000) 3887–3895.