Biochimica et Biophysica Acta 1833 (2013) 3254–3264

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Snf1/AMPK promotes SBF and MBF-dependent transcription in budding yeast

Sara Busnelli [a,b,1], Farida Tripodi [a,b], Raffaele Nicastro [a,b], Claudia Cirulli [a,b], Gabriella Tedeschi [c], Roberto Pagliarin [d], Lilia Alberghina [a,b,*], Paola Coccetti [a,b,*]

[a] SYSBIO, Centre of Systems Biology, Milan, Italy
[b] Department of Biotechnology and Biosciences, University of Milano-Bicocca, P.zza della Scienza 2, 20126 Milan, Italy
[c] D.I.P.A.V.-Biochemistry, University of Milano, Via Celoria 10, 20133 Milan, Italy
[d] Department of Chemistry, University of Milano, Via Golgi 19, 20133 Milan, Italy

ARTICLE INFO

Article history:
Received 6 May 2013
Received in revised form 20 September 2013
Accepted 23 September 2013
Available online 29 September 2013

Keywords:
Cell cycle
RNA Polymerase II
Saccharomyces cerevisiae
SBF–MBF complexes
Snf1/AMPK
SNF1^{as}(*I132G*)

ABSTRACT

Snf1, the yeast AMP-activated kinase homolog, regulates the expression of several genes involved in adaptation to glucose limitation and in response to cellular stresses. We previously demonstrated that Snf1 interacts with Swi6, the regulatory subunit of SBF and MBF complexes, and activates *CLB5* transcription. Here we report that, in α-factor synchronized cells in 2% glucose, the loss of the Snf1 catalytic subunit impairs the binding of SBF and MBF complexes and the subsequent recruitment of the FACT complex and RNA Polymerase II to promoters of G1-genes. By using an analog-sensitive allele of *SNF1*, *SNF1*^{as}(*I132G*), encoding a protein whose catalytic activity is selectively inhibited *in vivo* by 2-naphthylmethyl pyrazolopyrimidine 1, we show that the inhibition of Snf1 catalytic activity affects the expression of G1-genes causing a delayed entrance into S phase in cells synchronized in G1 phase by α-factor treatment or by elutriation. Moreover, Snf1 is detected in immune complexes of Rpb1, the large subunit of RNA Polymerase II, and is present at both promoters and coding regions of SBF- and MBF-regulated genes 20 min after α-factor release, suggesting a direct role for Snf1 in the activation of the G1-regulon transcription.

1. Introduction

The AMP-activated protein kinase (AMPK) family is constituted by Serine/Threonine protein kinases that are highly conserved in eukaryotes, from yeast and insects to plants and mammals. Their primary role is the integration of signals from nutrient availability and environmental stresses, to ensure the adaptation to those conditions and cell survival [1,2]. Like its homolog AMPK, the *Saccharomyces cerevisiae* Snf1 exists as a heterotrimeric complex made by the catalytic α subunit and the regulatory β and γ subunits [3,4]. The catalytic α subunit (encoded by *SNF1* gene) is activated by the phosphorylation of a T-loop threonine residue (Thr210) by one of the upstream kinases Sak1, Tos3 or Elm1 [5,6]. Snf1 activation also requires its interaction with the γ subunit Snf4, which stabilizes the active conformation of the kinase [7,8]. The interaction with one of the three alternative β subunits (encoded by either *SIP1*, *SIP2* or *GAL83* genes) determines the intracellular localization of Snf1 complex, targeting the kinase to specific substrates [9–11].

Snf1 upstream kinases are constitutively active, but metabolic signals, such as high glucose concentrations, regulate the protein phosphatase 1 complex (PP1) Reg1/Glc7, which dephosphorylates and hence inactivates Snf1 [12]. PP2A-type phosphatases have also been involved in regulating Snf1 activity [13,14]. The activation of Snf1 is finely regulated and various mutants have been reported to severely affect Snf1 kinase activity [15–20].

Although Snf1 is not allosterically activated by AMP as AMPK is [21], its activation is influenced by ADP which binds the γ subunit Snf4 and regulates the Reg1/Glc7 complex preventing Snf1 dephosphorylation [22,23].

Besides its well known role in metabolism, stress response and regulation of transcriptional activity of the histone acetyltransferase Gcn5 [4,24,25], a new function for Snf1 in the regulation of the G1/S phase transition of the cell cycle has been reported [26]. During this transition several metabolic and environmental signals are integrated and S-phase entrance requires a burst of transcription of about 200 genes (G1-regulon) [27–32], which is mediated by the transcription factors SBF and MBF. Both are heterodimers composed of a common regulatory subunit, Swi6, and two DNA-binding proteins, Swi4 in SBF and Mbp1 in MBF. SBF complex regulates transcription of genes involved in bud emergence and spindle pole body duplication, among which *CLN1*,

* Corresponding authors at: SYSBIO, Centre of Systems Biology, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Italy. Tel.: +39 02 64483521; fax: +39 02 64483519.
E-mail addresses: lilia.alberghina@unimib.it (L. Alberghina), paola.coccetti@unimib.it (P. Coccetti).
[1] Present address: European Institute of Oncology; IFOM-IEO Campus, via Adamello 16, Milan, Italy.

0167-4889/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamcr.2013.09.014

CLN2 and *PCL1* whereas MBF complex targets genes involved in DNA replication, repair and metabolism such as *CLB5*, *CLB6* and *RNR1* [33]. Although each factor controls a specific set of genes, a considerable functional overlap between SBF and MBF is reported [27,34–38].

SBF and MBF complexes play a major role in the control of G1 transcription [27,39–41] together with the chromatin remodeling complex FACT (FAcilitating Chromatin Transcription) and the Srb/mediator complex [42–47]. Activation of Cdk1/Cln and Pho85/Pcl complexes, by relieving Whi5 inhibition, allows the recruitment of RNA Polymerase II (RNA Pol II) and several other general transcription factors [29,48–50], which ultimately lead to the formation of a complete pre-initiation complex (PIC) at G1-genes [45,46,51,52].

In this study we have identified a new role for Snf1, revealing a previously unrecognized mode of regulation for G1-specific transcription in 2% glucose condition, which depends on its catalytic activity.

2. Materials and methods

2.1. Yeast strains and growth conditions

S. cerevisiae strains used in this study are listed in Table 1. Standard genetic methods were used for strain construction. Synthetic medium contained 2% glucose, 6.7 g/L of Yeast Nitrogen Base (Difco), 100–50 mg/L of required nutrients, at standard pH (5.5). Cell density of liquid cultures grown at 30 °C was determined with a Coulter counter on mildly sonicated and diluted samples. For G1 synchronization, cells were grown to exponential phase and α-factor (GenScript) was added to a final concentration of 3 μM. For experiments with the *SNF1(I132G)*^{as} strain, 25 μM 2NM-PP1 (from a 25 mM stock in 100% DMSO) was added 1 h before the release from α-factor to inhibit the activity of Snf1 [53] and 25 μM 2NM-PP1 was also added in the release medium. As a control, a culture grown in the presence of the same concentration of solvent (0.1% DMSO) was used.

Elutriations were performed essentially as in [54], using a 40 ml chamber elutriator (Beckman Coulter, Milan, Italy). Percentage of budded cells was determined by direct microscopic counting of at least 300 cells after mild sonication.

Table 1
Yeast strains used in this study.

Strain	Genotype	Source
Wt	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
snf1Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 snf1::KAN*	[26]
SNF1-myc	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SNF1-9myc:URA3*	[26]
SNF1-myc mbp1Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, mbp1::kanMX4 SNF1-9myc:URA3*	This study
SNF1-myc swi4Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, swi4::kanMX4 SNF1-9myc:URA3*	This study
SNF1-myc swi6Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0, swi6::kanMX4 SNF1-9myc:URA3*	This study
SWI4-myc	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SWI4-9myc:HPH*	This study
SWI4-myc snf1Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 SWI4-9myc:HPH snf1::KAN*	This study
MBP1-HA	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MBP1-4HA:URA3*	This study
MBP1-HA snf1Δ	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 MBP1-4HA:URA3 snf1::HPH*	This study
SNF1^{as}	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 snf1::KAN [pSNF1-I132G-3HA (URA3)]*	This study
SNF1-HA	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 snf1::KAN [pSNF1-3HA (URA3)]*	[26]
SNF1-T210A-HA	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 snf1::KAN [pSNF1-T210A-3HA (URA3)]*	[26]

All strains are isogenic relative to BY4741.

2.2. Protein extraction, immunoprecipitation assays and immunoblotting

Cells were collected by filtration and immediately frozen at −80 °C. Protein extractions, immunoprecipitations and western blot analysis were performed essentially as described in [26]. For co-immunoprecipation experiments, Swi6, Snf1-myc and RNA Pol II were immunopurified from 1 to 5 mg of total protein extract. Anti-HA mouse monoclonal antibody (12CA5, Roche), anti-myc mouse monoclonal antibody (9E10, Santa Cruz Biotechnology), anti-Swi6 rabbit polyclonal antibody (kindly provided by L.L. Breeden), anti-pThr210 Snf1 rabbit polyclonal antibody (kindly provided by S. Hohmann), anti-RNA Pol II (8WG16, Abcam) and anti-Pgk1 mouse monoclonal antibody (Molecular Probes) (1:500 dilution for anti-myc; 1:1000 dilution for all other antibodies) were used for western blot analysis.

2.3. Flow cytofluorimetric analysis

Cells were collected, fixed in 70% ethanol, stained with SYTOX Green nucleic acid stain (Molecular Probes) and analyzed for DNA content as described in [26]. Flow cytofluorimetric analysis was performed using a BD FACScan (Becton-Dickinson).

2.4. RNA purification and qReal-time PCR

Total RNA was isolated using a phenol-chloroform protocol, essentially as previously described [26]. Reverse transcription of 0.5 μg of mRNAs was carried out with iScript cDNA Synthesis Kit (BIO-RAD). Quantitative Real-time PCR for *CLN2*, *PCL1*, *CLB5* and *RNR1* gene expression was performed using SsoFast EvaGreen Supermix (BIO-RAD), oligos available upon request. Obtained data were normalized to both *PGK1* and *CDC34* reference genes and organized with CFX manager software (BIO-RAD). Data are presented as the mean value ± standard deviation from at least three independent experiments performed in triplicate.

2.5. Chromatin Immunoprecipitation and sequential chromatin immunoprecipitation

Chromatin Immunoprecipitation was performed essentially as previously described in [55]. In a few instances the protocol was changed as described afterward. Cells were grown and treated with 1% formaldehyde for 25 min. Glycine was added to a final concentration of 375 mM and the cultures were further incubated for 5 min at room temperature. Cells were then washed once with cold HBS buffer (50 mM Hepes pH 7.5, 140 mM NaCl); after filtration cell pellets were resuspended in 400 μl ChIP lysis buffer (50 mM Hepes pH 7.5, 140 mM NaCl, 1 mM EDTA, 0.1% sodium deoxycholate, 1% Triton X-100, 1 mM PMSF, proteases inhibitor mix Complete EDTA free Protease Inhibitor Cocktails Tablets, Roche). The cell suspension was then mixed with an equal volume of glass-beads and cells were broken at 4 °C on vortex. Cell lysates were sonicated to yield an average DNA fragment size of 500 bp and clarified by centrifugation at 10,000 ×*g* for 15 min. Protein concentration for each sample was measured by the Bradford method using a BIO-RAD protein assay kit. An equal amount of proteins (0.5–1 mg) was incubated overnight at 4 °C with 5 μl of anti-myc mouse monoclonal antibody (9E10, Santa Cruz Biotechnology), anti-HA mouse monoclonal antibody (12CA5, Roche), anti-Swi6 rabbit polyclonal antibody (kindly provided by L.L. Breeden), anti-Spt16 antibody (kindly provided by T. Formosa), anti-Rpb1 antibody (8WG16, Abcam) and 30 μl of protein A magnetic beads (Millipore) previously equilibrated in ChIP lysis buffer. The beads-bound immunocomplexes were washed three times with ChIP lysis buffer, twice with TBS (20 mM Tris–HCl pH 7.5, 150 mM NaCl) and once with TE (10 mM Tris–HCl pH 8.0, 1 mM EDTA). Each wash was performed for 10 min at 4 °C. To detect Snf1-myc at *CLN2* and *RNR1* genes a sequential ChIP was performed, starting with a ChIP with anti-Rpb1 antibody (1st ChIP) followed by a ChIP

using anti-myc antibody (2nd ChIP). For both ChIP and sequential ChIP, the immunoprecipitated material was eluted from magnetic beads by incubation at 65 °C for 10 min with 150 μl of TE containing 1% SDS. The eluted material was then incubated at 65 °C for 6 h to reverse the cross-link and the immunoprecipitated DNA was purified using a PCR purification kit (Qiagen) following the instructions of the manufacturer. Control immunoprecipitations without anti-RNA Pol II antibody (*no Ab*) or from a strain without tag (*no tag*) were also performed to subtract the background. For the sequential ChIP assay, as a control for the first ChIP anti-Rpb1 antibody was omitted in the *no Ab* control; for the second ChIP in the *no Ab* control, anti-myc antibody was omitted. Real-time PCRs were performed to quantify the relative enrichment of target DNA fragments after immunoprecipitation with anti-myc, anti-HA or anti-RNA Pol II antibody. Reactions were performed in triplicate using the Sso Fast EvaGreen Supermix (BIO-RAD) and carried out in a MiniOpticon (BIO-RAD). The enrichment of target regions were calculated using an intergenic region of Chromosome I as a reference for nonspecific DNA, as reported in [56]. For each experiment shown, ChIP analysis was performed at least twice using independently prepared batches of chromatin. The results were compared by using a two-sided Student's *t*-test. Differences were considered statistically significant at $p < 0.05$.

2.6. Chemical synthesis

Synthesis and spectroscopic data of compound 2NM-PP1 are reported in Supplementary information.

3. Results

3.1. Snf1 promotes transcription of G1-specific genes

Previous studies from our group have revealed a connection between protein kinase Snf1 and cell cycle control at the G1/S phase transition, since loss of Snf1 affects the proper expression of *CLB5* gene [26]. To further investigate whether Snf1 could be involved in the regulation of other G1-regulon genes, we extended our analyses to both SBF- and MBF-dependent genes. As shown in Fig. S1A, *SNF1* deletion reduced growth rate in synthetic medium with 2% glucose as carbon source, as previously reported [26], with about 43% of the population which shows 1C DNA content (Supplementary Fig. S1B) and a reduced expression of G1-specific genes (Supplementary Fig. S1C).

To better investigate this phenotype in 2% glucose, *snf1Δ* cells were synchronized by α-factor treatment and the expression of two representative SBF-dependent genes (*CLN2* and *PCL1*) and two MBF-dependent ones (*CLB5* and *RNR1*) was investigated. As shown in Fig. 1 the expression of those genes was reduced mainly 20 min after α-factor release, coherently with the delayed S-phase entrance (previously reported in [26]), indicating that Snf1 may be involved in the regulation of both SBF- and MBF-dependent transcription.

The induction of G1-specific transcription requires the transcription factor Swi6, which is tethered to promoters through its binding to Swi4 and Mbp1 [34,57]. Given that Snf1 interacts with Swi6 [26] also in cells lacking the DNA-binding proteins Swi4 or Mbp1 (Fig. 2A), we examined, by ChIP experiments, whether Snf1 was able to affect the interaction of SBF and MBF subunits to promoters of *CLN2* and *CLB5* genes in G1-synchronized cells. In *snf1Δ* mutant, after α-factor release, Swi6 binding to *CLN2* and *CLB5* promoters was impaired, remaining low and without any significant increase at all analyzed time points (Fig. 2B). On the contrary wild type cells showed an increase of Swi6 binding to *CLN2* and *CLB5* promoters 10–20 min after the release (Fig. 2B), coherently with the maximum expression of the corresponding mRNAs (Fig. 1).

It is well known that Swi6 interaction to promoters of G1-genes is mediated by the DNA binding-proteins Swi4 and Mbp1 [36,58,59]. Since our data showed a defective Swi6 recruitment to promoters in the *snf1Δ* strain, we investigated whether that alteration could reflect a dysfunctional interaction of Swi4 and Mbp1 to DNA. We therefore performed ChIP analyses in G1-synchronized cells in order to evaluate the binding of Swi4 to SCB elements of *CLN2* promoter and of Mbp1 to MCB elements of *CLB5* promoter. Our analyses showed that in the control culture, *in-locus* tagged Swi4-myc and Mbp1-HA proteins were recruited to promoters and their binding increased and reached a maximum 10 min after the α-factor release (Fig. 2C and D). On the contrary, *SNF1* deletion impaired the interaction of both Swi4-myc and

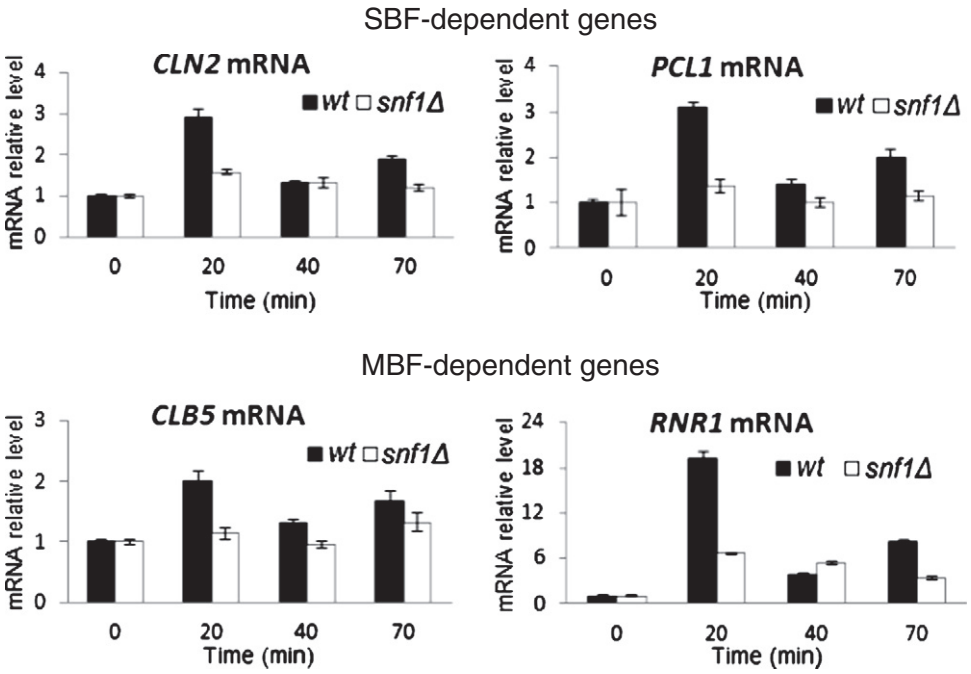

Fig 1. Snf1 deletion affects G1-gene transcription. wt and *snf1Δ* strains were grown in synthetic medium containing 2% glucose until exponential phase, G1-arrested by α-factor treatment and released into fresh medium. Samples were taken at the indicated time points to assay *CLN2*, *PCL1*, *CLB5* and *RNR1* mRNA level by quantitative relative Real-time PCR. Reported values are the mean ± standard deviations of three independent experiments.

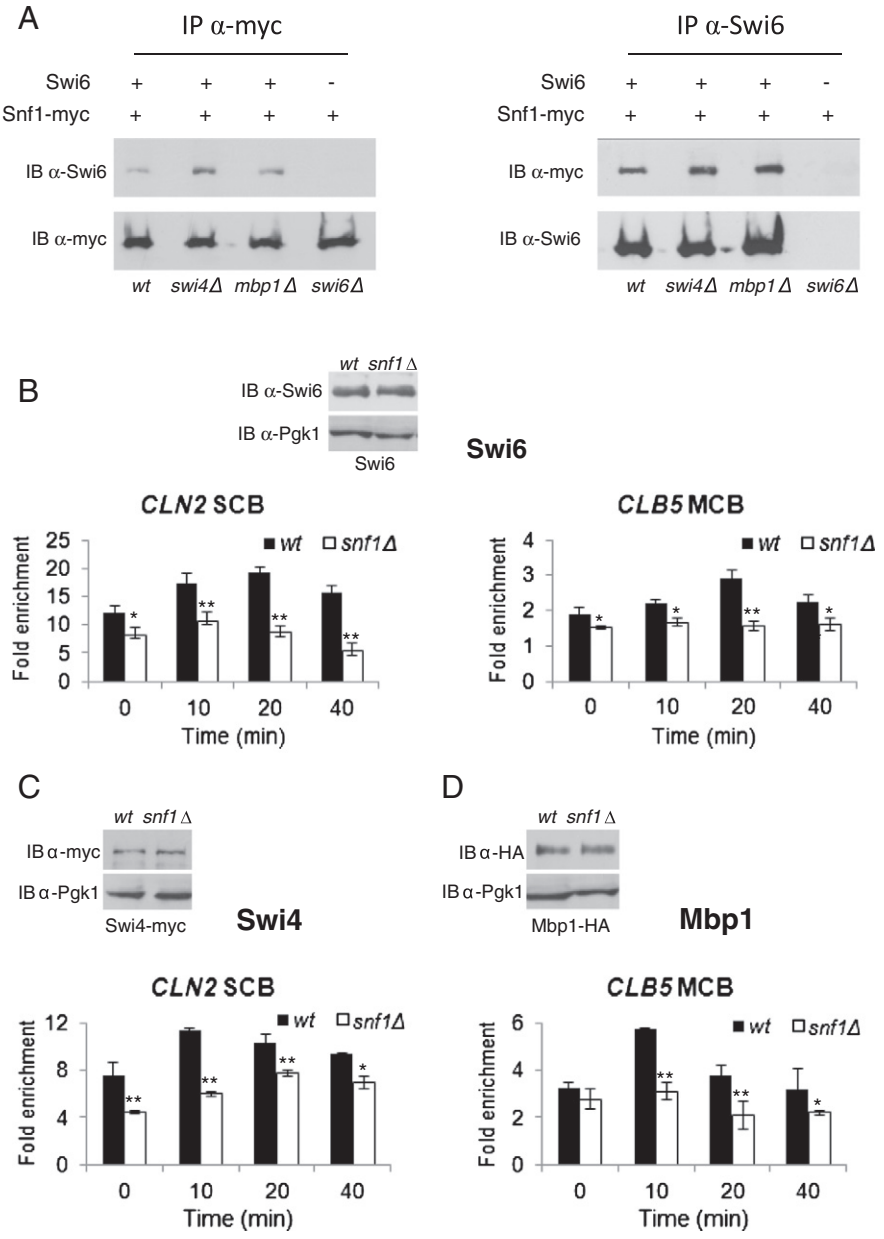

Fig. 2. Snf1 is required for the binding of SBF and MBF complexes to G1-promoters. (A) The strains wt, *swi4Δ*, *mbp1Δ* and *swi6Δ* expressing *in-locus* tagged Snf1-myc were grown in synthetic medium containing 2% glucose, the immunoprecipitations (IP) were performed with anti-myc antibody (left panel) or anti-Swi6 antibody (right panel) and analyzed by western blot (IB) with anti-Swi6 antibody and anti-myc antibody. (B) wt and *snf1Δ* strains were grown in synthetic medium containing 2% glucose, G1-arrested by α-factor treatment and released. Samples were collected at the indicated time points after the release to perform a Chromatin Immunoprecipitation (ChIP) analysis using anti-Swi6 antibody. (C–D) wt and *snf1Δ* strains expressing *in-locus* tagged Swi4-myc (C) or Mbp1-HA (D) were grown in synthetic medium containing 2% glucose, synchronized in G1-phase by α-factor treatment and released. Samples were collected at the indicated time points after the release to perform ChIP analysis using anti-myc or anti-HA antibodies. (B–D) Specific regions of *CLN2* and *CLB5* genes promoter containing SCB or MCB elements were amplified. The amount of immunoprecipitated DNA was normalized to a non-transcribed region of Chromosome I sequence present in the IP DNA and in consideration of the input DNA. Relative quantification was performed with respect to a *no tag* strain control, which was set as 1. $^{*}p < 0.05$, $^{**}p < 0.005$. Reported values are the mean ± standard deviations of at least two independent experiments. Insets show the amount of Swi6-myc, Swi4-myc and Mbp1-HA proteins in wt and *snf1Δ* strains, analyzed by western blot with anti-myc or anti-HA antibody. Pgk1 was used as a loading control.

Mbp1-HA to *CLN2* and *CLB5* promoters, without affecting their protein level (Fig. 2C and D).

Taken together, these findings support the notion that the binding of SBF and MBF components to G1-promoters is lower in *snf1Δ* cells.

3.2. Snf1 is required to recruit FACT complex and RNA Pol II to G1-gene promoters

The binding of SBF and MBF complexes to DNA sets the stage for the interaction of co-activators, including the FACT complex, which regulate chromatin remodeling to guarantee transcriptional initiation [46,47]. The co-activators, then, promote the binding of RNA Pol II to DNA, an event which determines the formation of a Pre-Initiation Complex (PIC) required for transcription initiation [43–45,47].

Since our data indicate that *SNF1* deletion is consistent with a reduction of SBF and MBF binding to *CLN2* and *CLB5* promoters, we investigated whether this effect could influence the formation of the Pre-Initiation Complex. For this reason, we analyzed the binding of the FACT complex and of RNA Pol II to G1-specific gene promoters. Wild type and *snf1Δ* strains were synchronized in G1 phase by α-factor

treatment and released into fresh medium. Then, a ChIP assay was performed using an antibody specific for Spt16 (a component of FACT complex) or for Rpb1 protein (the large subunit of RNA Pol II complex). Immunopurified DNA was analyzed by Real-time PCR using primers which amplify TATA box sequences of *CLN2*, *PCL1*, *CLB5* and *RNR1* promoters. ChIP analyses performed in the control strain showed, as expected, a peak of the FACT binding at promoters 20 min after the release from α-factor arrest (Fig. 3A). On the contrary, in the *snf1Δ* strain no peak of Spt16 recruitment was observed at any analyzed promoter (Fig. 3A). In keeping with those data, also the association of RNA Pol II to the same promoters was impaired in the *snf1Δ* mutant (Fig. 3B).

Therefore, our findings indicate that the formation of the Pre-Initiation Complex, which is required for the transcription initiation of G1-specific genes, is affected in cells lacking *SNF1*.

3.3. Snf1 kinase activity is required for the transcription of G1-specific genes

Since our data indicate a function of Snf1 in cells grown in not limiting glucose conditions (2% glucose), we analyzed whether that role might depend on its kinase activity. First of all, a slight phosphorylation of Thr210 residue of Snf1 was detectable in 2% glucose, suggesting that in this condition Snf1 kinase was partially catalytically active (Fig. 4A), consistent with previous reported data [60,61]. Then, to investigate the function of Snf1 kinase activity on the expression of cell cycle genes, we used a strain expressing a non-phosphorylatable form of Snf1 (Snf1-T210A), which cannot be activated by phosphorylation on the T-loop Thr210 residue [62]. As previously reported [26], this strain shows a delay of S-phase entrance, although to a lesser extent than a *snf1Δ* strain. Coherently, the expression of SBF- and MBF-dependent genes was reduced mainly 20 min after α-factor release (Fig. 4B).

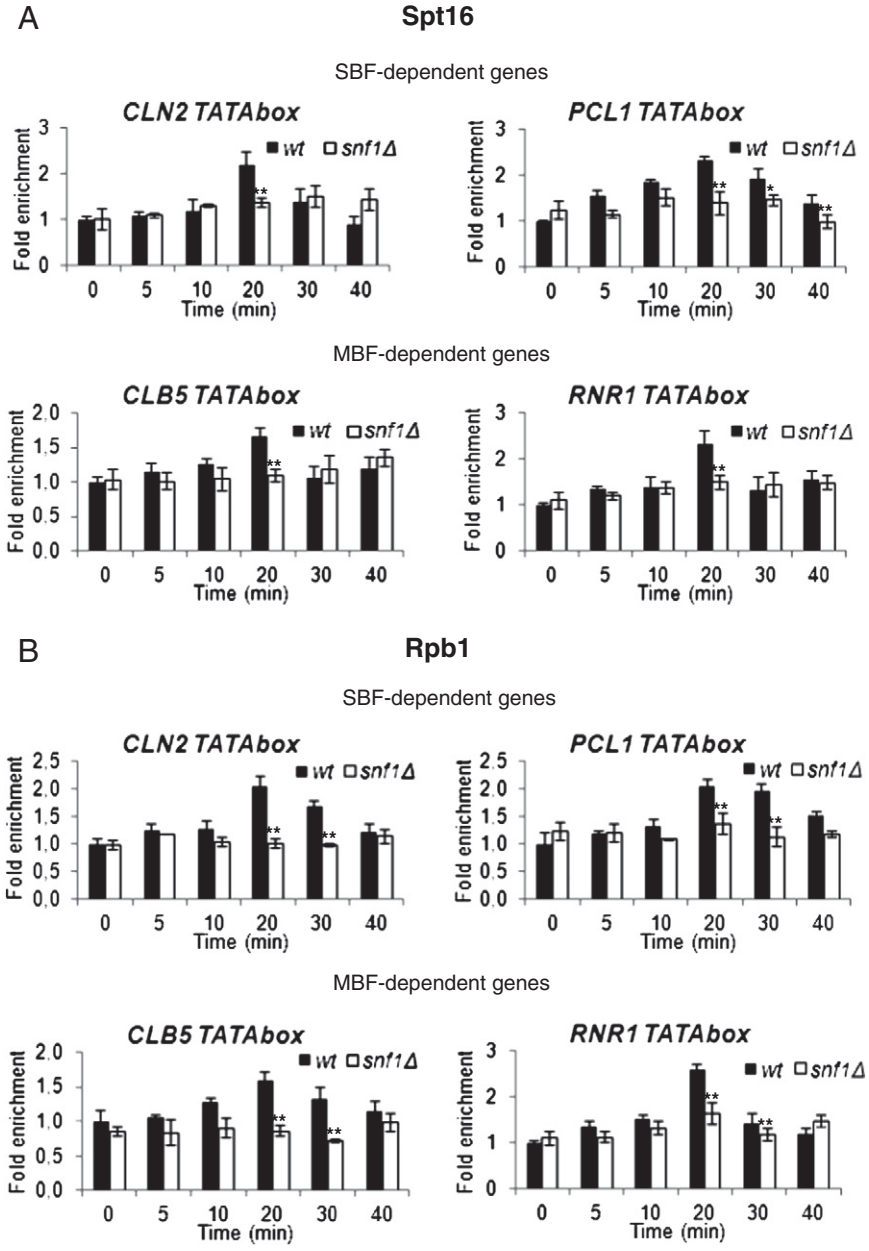

Fig. 3. Snf1 is required for RNA Pol II and FACT complex recruitment to SBF- and MBF-dependent promoters. (A–B) wt and *snf1Δ* strains were grown in exponential phase, G1-arrested by α-factor treatment and released into fresh medium. Samples were taken at the indicated time points to perform Chromatin Immunoprecipitation assay using anti-Spt16 antibody or anti-Rpb1 antibody (8WG16). Relative Spt16 (A) or Rpb1 (B) occupancy at the TATA box of *CLN2*, *PCL1*, *CLB5* and *RNR1* genes was set with respect to occupancy of wild type strain at time 0 min, which was set as 1. Reported values are the mean ± standard deviations of two independent experiments, all data were normalized to a non-transcribed region of Chromosome I. *$p < 0.05$, **$p < 0.005$.

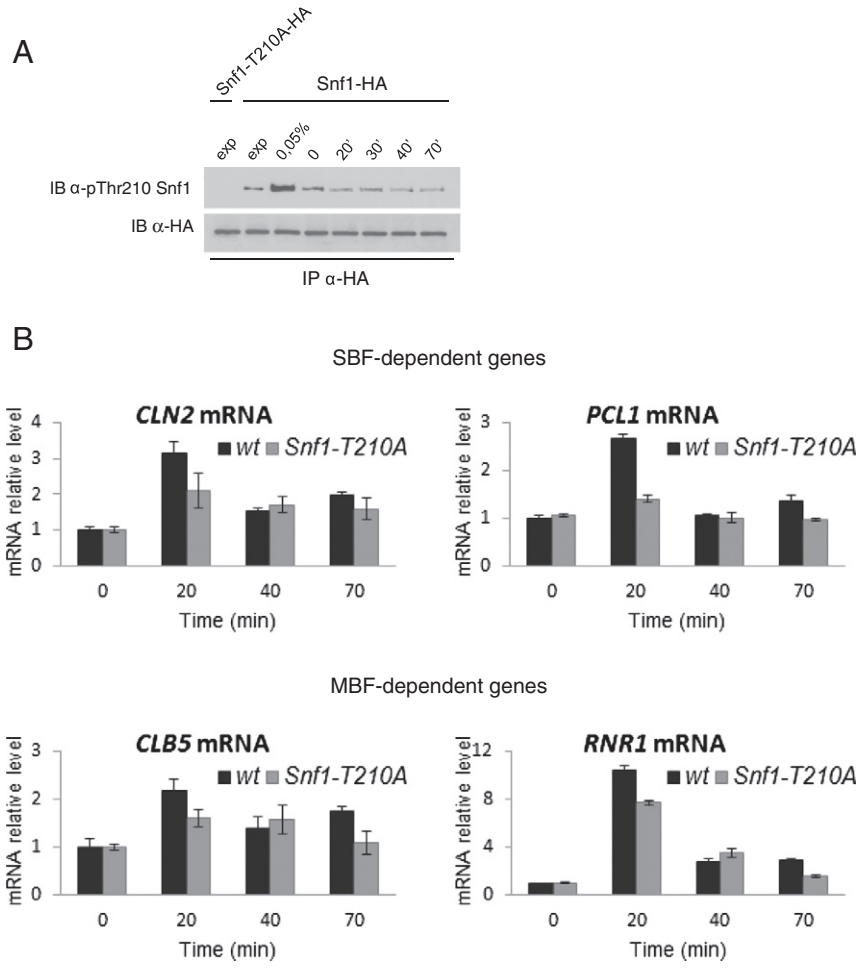

Fig. 4. Snf1 is phosphorylated on residue T210 in 2% glucose growing cells. (A) A Snf1-HA expressing strain was grown in synthetic medium containing 2% glucose until exponential phase, then a part of the culture was filtered, washed and shifted into synthetic medium containing 0.05% glucose for 10 min (0.05%). The rest of the culture was synchronized in G1 phase by α-factor treatment, released into fresh medium and samples were taken at different time points (0, 20, 30, 40, 70 min). Snf1-HA was immunoprecipitated (IP) with anti-HA antibody and analyzed by western blot (IB) with anti-pT210 Snf1 antibody or anti-HA antibody. As a control of antibody specificity, Snf1 was immunoprecipitated from a strain expressing Snf1-T210A-HA. (B) Strains expressing Snf1 wt or Snf1-T210A were grown in synthetic medium containing 2% glucose until exponential phase, G1-arrested by α-factor treatment and released into fresh medium. Samples were taken at the indicated time points to assay *CLN2*, *PCL1*, *CLB5* and *RNR1* mRNA level by quantitative relative Real-time PCR. Reported values are the mean ± standard deviations of three independent experiments.

Since it was previously reported that *SNF1-T210A* mutation did not completely abolish Snf1 activity [15,16,18] we used a strain expressing an analog-sensitive (as) allele of *SNF1*, $SNF1^{as}$(*I132G*) encoding a protein whose catalytic activity is selectively inhibited *in vivo* by 2-naphthylmethyl pyrazolopyrimidine 1 (2NM-PP1) [53].

Treatment with 2NM-PP1 reduced the growth rate of exponentially growing $SNF1^{as}$ cells, determining an increase of cells with 1C DNA content, already detectable 60 min after the treatment (Supplementary Fig. S2), rendering the $SNF1^{as}$ strain very similar to a *snf1Δ* strain (compare Supplementary Fig. S2 with Supplementary Fig. S1).

Then, to investigate this effect on a synchronous population, exponentially growing $SNF1^{as}$ cells were synchronized with α-factor, after 1 h the culture was treated with 25 μM 2NM-PP1 for an additional hour and then released into fresh medium containing 25 μM 2NM-PP1. In the absence of the inhibitor, in accordance with the onset of bud emergence and DNA replication, the $SNF1^{as}$ strain expressed G1-specific genes as the wild type (Fig. 5A–C). Remarkably, the addition of 2NM-PP1 delayed S-phase entrance by affecting the expression of both SBF- and MBF-dependent genes (Fig. 5A–C) as well as the association of Rpb1 to *CLN2*, *PCL1*, *CLB5* and *RNR1* promoters (Fig. 5D). As expected, treatment with 2NM-PP1 on *SNF1wt* cells had no effect on budding index, DNA content and G1-gene expression (Supplementary Fig. S3), confirming the specificity of 2NM-PP1.

To exclude any artefacts due to the use of α-factor in our experiment, centrifugal elutriation was chosen as a different method of G1 synchronization. After release in fresh pre-warmed medium, G1 elutriated *snf1Δ* cells remained mostly unbudded until 240 min, indicating that loss of Snf1 causes a severe delay in the beginning of a new cell cycle (Supplementary Fig. S4). Nevertheless, since it is well known that centrifugation activates Snf1 [21], we decided to overcome any problem due to centrifugation in a *snf1Δ* background making the same experiment on $SNF1^{as}$ cells. $SNF1^{as}$ cells were grown in synthetic medium with 2% glucose until late exponential phase and elutriated to obtain cells in G1 phase in the absence of the inhibitor, to ensure that cells with an active Snf1 protein could respond to the *g* stress of centrifugation. Small unbudded cells were then re-inoculated in fresh pre-warmed medium at time 0 with 0.1% DMSO (solvent control) or with 2NM-PP1 to inhibit Snf1 activity. As reported in Fig. 6, treatment with the inhibitor severely impaired budding, DNA replication, as well as the expression of *CLN2* and *RNR1* mRNA (Fig. 6A–C), with an increase of *CLN2* expression only 135 min after the release (Fig. 6C).

Taken together our results strongly support the notion that the catalytic activity of Snf1 is required to promote G1-transcription.

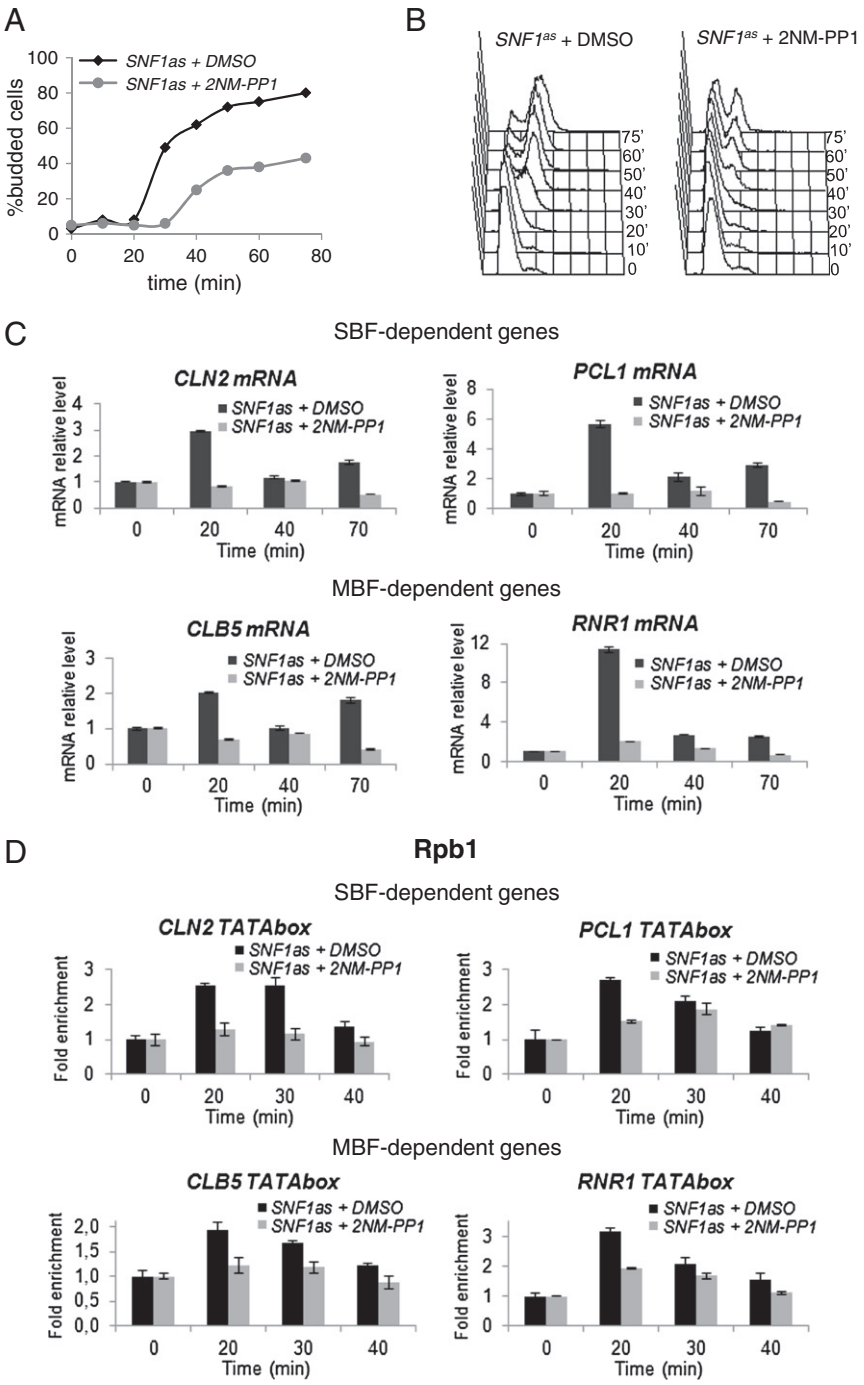

Fig. 5. Snf1 catalytic activity is required to promote G1-gene transcription. *SNF1*^{as} strain was grown in synthetic medium containing 2% glucose until exponential phase and G1-arrested by α-factor treatment. After 1 h, the culture was split in two: half of the culture was treated with 0.1% DMSO and the rest of the cells was treated with 25 μM 2NM-PP1 (final concentration of DMSO 0.1%) for an additional hour. Then the cultures were released in fresh medium containing 0.1% DMSO or 25 μM 2NM-PP1. Samples were taken at the indicated time points to assay (A) budding index, (B) DNA content by FACS analysis and (C) *CLN2*, *PCL1*, *CLB5* and *RNR1* mRNA level by quantitative relative Real-time PCR. Reported values are the mean ± standard deviations of three independent experiments. (D) Chromatin Immunoprecipitation assay using anti-Rpb1 antibody (8WG16). Relative Rbp1 occupancy at the TATA box of *CLN2*, *PCL1*, *CLB5* and *RNR1* genes was set with respect to occupancy of wild type strain at time 0 min which was set as 1. Reported values are the mean ± standard deviations of two independent experiments, all data were normalized to a non-transcribed region of Chromosome I.

3.4. Snf1 is recruited to both SBF and MBF-regulated promoters

Since Snf1 modulates the recruitment of RNA Pol II to promoters, a physical interaction of Snf1 with RNA Pol II was investigated. We found that Snf1 was detected in Rpb1 immune complexes 20 min after α-factor release (Fig. 7A).

We reasoned therefore that Snf1 itself could be directly recruited to the promoters, together with RNA Pol II. Since it has recently been reported that Snf1 is detectable at the promoter of *ADY2* by a sequential ChIP approach [63], we performed a sequential ChIP experiment using an initial ChIP with anti-Rpb1 antibody and then a second re-ChIP with anti-myc antibody to enrich for chromatin bound to Snf1. A strain expressing an *in-locus* tagged Snf1-myc protein was synchronized in G1 phase by α-factor treatment and released into fresh medium; samples were collected at times 0 and 20 min after the release for sequential ChIP experiments (considering that the maximum recruitment of RNA Pol II to promoters was detected at 20 min, as shown in Fig. 3B). Immunopurified DNA was analyzed by Real-time PCR using primers

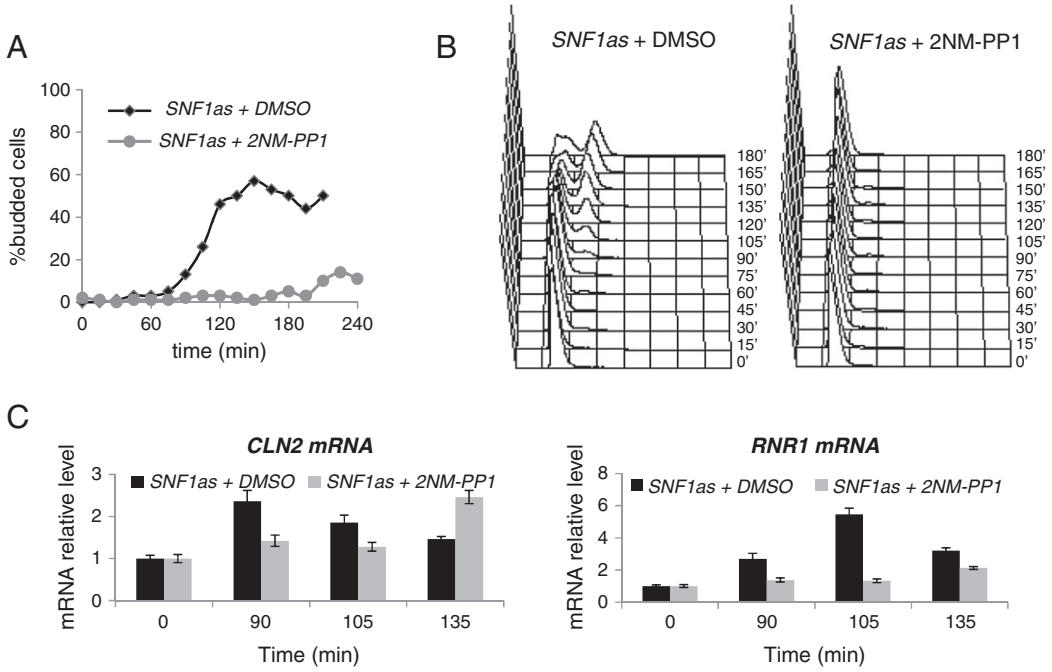

Fig. 6. Snf1 is required for G1-gene expression in elutriated cells. *SNF1*^{as} strain was grown in synthetic medium containing 2% glucose until late exponential phase, elutriated to obtain cells in G1 phase that were then released in fresh pre-warmed medium containing 0.1% DMSO or 25 μM 2NM-PP1. Samples were taken at the indicated time points to assay (A) budding index, (B), DNA content by FACS analysis, and (C) *CLN2* and *RNR1* mRNA level by quantitative relative Real-time PCR.

which amplified TATA box sequences and two internal regions inside *CLN2* and *RNR1* genes (Int. 1 and Int. 2, amplicon positions shown in Fig. 7B and C). Both *CLN2* and *RNR1* promoter sequences were specifically detected in association with Snf1-myc 20 min after the release from G1 arrest (Fig. 7B and C, compare the increase of signal between the first and the second ChIP in Snf1-myc cells *vs no tag* control). Furthermore, capture of Snf1-myc specifically recovered also DNA fragments of internal coding regions of both genes (Fig. 7B and C). No association of Snf1-myc was detected at *CDC34* gene (Supplementary Fig. S5), supporting the specificity of the presence of Snf1 at SBF- and MBF-dependent genes.

Collectively these data indicate that the catalytic activity of Snf1 modulates G1-specific transcription by promoting the interaction of RNA Pol II to SBF- and MBF-regulated genes.

4. Discussion

Accurate and regulated transcription of eukaryotic genes represents a major step in gene regulation, requiring the coordinated activity of a large number of proteins and protein complexes. It is well known that Snf1, the master regulator of cellular energy balance [64], regulates transcription of various metabolic genes acting at different levels, including regulation of transcription factors [65–67], modulation of Chromatin remodeling process [25,63,68,69] and interaction with the Srb/mediator complex of RNA polymerase II holoenzyme [19].

Building upon previous results from our laboratory, which indicated that Snf1 positively regulates yeast cell cycle progression in cells growing in 2% glucose [26], we newly report in this paper a direct involvement of Snf1 catalytic activity in modulating different aspects of transcription at the G1-regulon. Indeed the mutant *SNF1*^{as} treated with the inhibitor 2NM-PP1 shows a reduced recruitment of RNA Pol II to G1 promoters and, as a consequence, a reduced transcription of G1-regulon which leads to a delayed S-phase entrance (Fig. 5–6). Notably, the binding of Swi6, Swi4 and Mbp1 to SCB and MCB elements is affected in the absence of Snf1 (Fig. 2) and Snf1 protein is detectable not only at the TATA boxes of SBF- and MBF-dependent promoters but also at the internal coding regions of the same genes (Fig. 7A–C), confirming the requirement of Snf1 activity for G1 transcription. Moreover, also the *SNF1-T210A* mutant has an impaired expression of G1-specific genes (Fig. 4B), although to a lesser extent than the *snf1Δ* strain. Consistently, we previously reported that while the *SNF1-T210A* mutant shows a cell-cycle defect in 2% glucose and a delayed S-phase entrance, the *SNF1-T210E* mutant is almost undistinguishable from the wild type strain [26], suggesting a constitutive phosphorylation of Snf1 on T210 in 2% glucose, as we show in the present paper (Fig. 4A). The fact that Snf1 is weakly phosphorylated also in 2% glucose confirms that it can be partially active in that growth condition. In keeping with our results there are multiple lines of evidence indicating regulatory roles for Snf1 basal kinase activity in the presence of glucose. In fact, previous studies showed that a low level of Snf1 activity is sufficient and essential to confer resistance to different stress conditions such as toxic cations, hydroxyurea and heat shock also in glucose growing cells [15,24,70]. Furthermore, it has also been reported that Snf1 stimulates Gcn2 kinase activity during histidine starvation of glucose grown-cells [71], reinforcing the notion of a quantitative regulation of Snf1 kinase activity.

Although the general observation is that the absence of Snf1 causes little or no perturbations of cells growing in complete medium (YPD, with 2% glucose), we highlight that *SNF1* deletion reduces growth rate in synthetic medium with 2% glucose, increases the percentage of cells with 1C DNA content and reduces the expression of G1-specific genes (Supplementary Fig. S1). By using the *SNF1*^{as} mutant we also show that treatment with 2NM-PP1 reduces the growth rate of exponentially growing *SNF1*^{as} cells, determining an increase of cells with 1C DNA content (Supplementary Fig. S2), rendering the *SNF1*^{as} mutant very similar to a *snf1Δ* strain and indicating that the kinase activity of Snf1 is important for cell growth also in non-synchronized cells. This phenotype is fully complemented in 5% glucose [26]. Our data therefore indicate a new function of Snf1 in glucose growing cells, although we believe that additional work is required to better elucidate that role.

One question that still needs to be answered is which are the substrates of Snf1 involved in G1-transcription. Remarkably, although Swi6 is phosphorylated by Snf1 *in vitro* on Ser760, site-specific mutants (*SWI6-S760A* or *SWI6-S760E*) show neither an alteration of growth rate

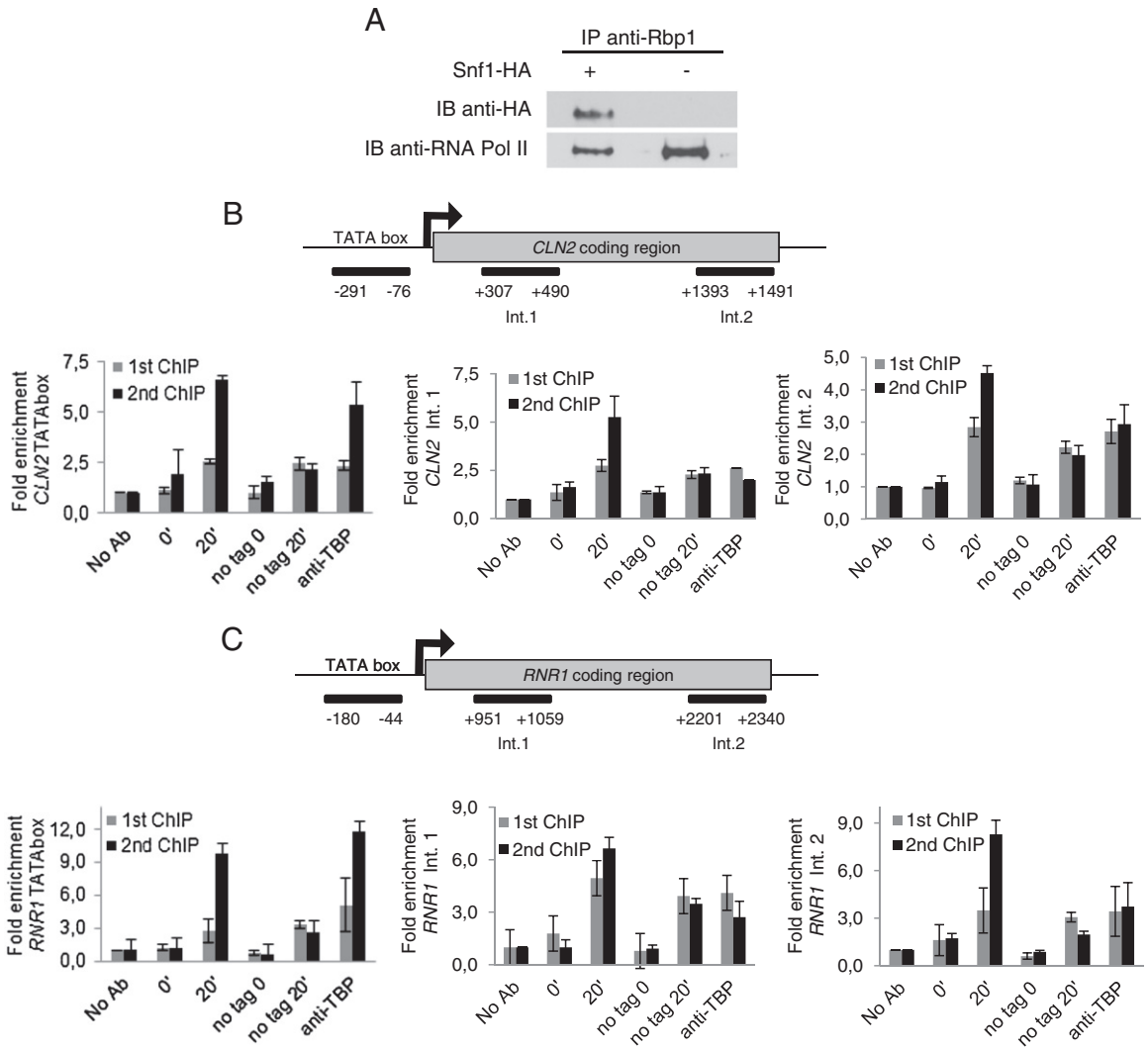

Fig. 7. Snf1 interacts with *CLN2* and *RNR1* genes. (A) A co-immunoprecipitation experiment was performed using a *SNF1-HA* strain, collected 20 min after α-factor release. The immunocomplexes (IP) were precipitated with anti-Rpb1 antibody (8WG16) and analyzed by immune-blot (IB) with anti-HA antibody and anti-Rpb1 antibody (8WG16). A *no tag* strain was used as a control. (B–C) Diagrams of *CLN2* and *RNR1* genes with PCR amplicones corresponding to TATA box and two internal regions downstream from the beginning of the genes. Snf1-myc and *no tag* wild-type cells were grown in synthetic medium containing 2% glucose, G1-arrested with α-factor treatment and released into fresh medium; samples were collected at the indicated time points after the release. Chromatin Immunoprecipitation analysis were performed using antibody against Rpb1 protein (8WG16 antibody) (1st ChIP), followed by immunoprecipitation with antibody anti-myc (2nd ChIP) to detect Snf1. DNA was amplified by Real-time PCR. To test the ability of the assay to efficiently detect protein binding at different regions along genes, immunoprecipitation was firstly performed with anti-Rpb1 antibody and then with anti-TATA Binding Protein (anti-TBP). Reported values are the mean ± standard deviations of two independent experiments. The amount of immunoprecipitated DNA was normalized to a non-transcribed region of Chromosome I sequence present in the IP DNA and in consideration of the input DNA. Relative quantification was performed with respect to a *no antibody* control, which was set as 1.

nor relevant defects in S-phase entrance (Supplementary Fig. S6), suggesting that Snf1 could stimulate gene expression by phosphorylating different key proteins, acting at several levels of the transcriptional process under physiological conditions. Notably, Snf1 is known to modulate the acetylation of histones at different genes [63,72] and a recent work has also suggested a possible role of this kinase on the activity of RNA Pol II complex [19]. Therefore, although we found Snf1 in immune complexes of Rpb1, we do not completely exclude an indirect interaction between Snf1 and RNA Pol II mediated by other components of the transcription apparatus.

As summarized in Fig. 8, novel findings obtained in this paper show that Snf1 plays a relevant role in regulating the G1/S transition by (*i*) promoting the binding of Swi4, Mbp1 and Swi6 proteins to SCB and MCB elements of G1 promoters and consequently (*ii*) favoring the proper recruitment of the FACT complex and RNA Pol II, which are required for both transcription initiation and elongation at the same genes; (*iii*) moreover our evidences show that Snf1 interacts with promoters and coding regions of G1-genes, thus a possible involvement in the elongation process can also be suggested.

Until now, the importance of Snf1/AMPK has been limited to its role as a regulator of cellular metabolism. However, a link between metabolic regulation and cell cycle control is required in order to ensure that cell division occurs only if the energy status of the cell is able to support it. Several data suggest the existence of such a link in higher eukaryotes, but this regulation appears to be complex. Literature data indicate that in mammalian cells the activation of AMPK correlates with the arrest of cell cycle in G1 phase [73,74]. On the other hand, studies in *Drosophila* and mammalian cells have demonstrated that the role of AMPK in energy sensing is coupled with essential cell biology functions, such as cell polarity and cell division [75]. AMPK also regulates mitotic progression in mammalian cells, independently from a low cellular energy status [76,77] and data from *Arabidopsis thaliana* indicate a function for Snf1-related protein kinases KIN10/KIN11 in normal vegetative and reproductive growth [78].

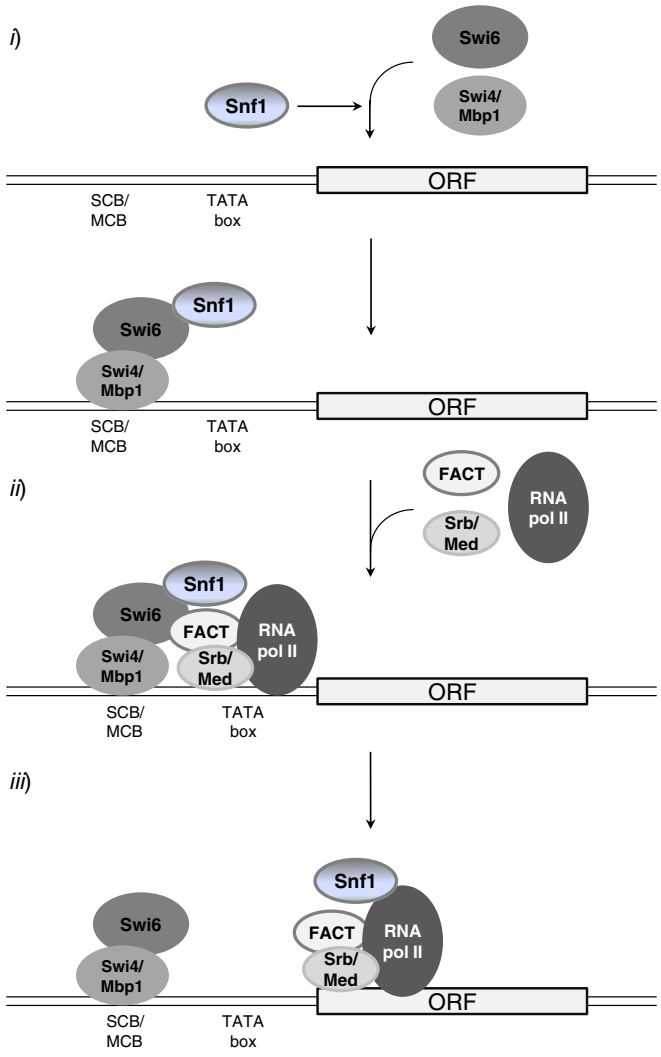

Fig. 8. A model of the regulatory role of Snf1 in G1 transcription. (*i*) Snf1 promotes the binding of Swi4, Mbp1 and Swi6 proteins to SCB and MCB elements of G1 promoters and (*ii*) favors the proper recruitment of the FACT complex and RNA Pol II, (*iii*) which are required for transcription initiation and elongation at the same genes. Snf1 association with promoters and coding regions of G1-genes suggests a possible involvement of Snf1 also in the elongation process. Snf1 association to DNA could be directly or indirectly mediated by RNA-pol II or by other key components of the transcription apparatus.

Thus, our novel results, which show a role for Snf1 in the regulation of cell cycle in not-limiting glucose conditions, indicate that in the budding yeast, as in higher eukaryotes, apart from the regulation of cellular processes in response to energy stresses, the conserved Snf1/AMPK family can coordinate essential and basic cellular functions under physiological conditions as well.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

We thank S. Hohmann for *pSNF1-3HA* plasmid and anti-pThr210 Snf1 antibody, L.L. Breeden for anti-Swi6 antibody, T. Formosa for anti-Spt16 antibody and K. M. Arndt for *pSNF1-I132G-3HA* plasmid. We thank M. Vanoni for the constructive comments and suggestions on the manuscript and Neil Campbell for the language editing.

This work was supported by a grant to P.C. (FAR 2010) and by grants to L.A. from the EU-funded project UNICELLSYS (http://www.unicellsys.eu/), from EU-funded Coordination Action Yeast Systems Biology Network (YSBN; http://www.ysbn.eu/) and from the MIUR-funded "SysBioNet" project of the Italian Roadmap for ESFRI Research Infrastructures.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamcr.2013.09.014.

References

[1] D.G. Hardie, AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy, Nat. Rev. Mol. Cell Biol. 8 (2007) 774–785.
[2] R. Ghillebert, E. Swinnen, J. Wen, L. Vandesteene, M. Ramon, K. Norga, F. Rolland, J. Winderickx, The AMPK/SNF1/SnRK1 fuel gauge and energy regulator: structure, function and regulation, FEBS J. 278 (2011) 3978–3990.
[3] N. Nath, R.R. McCartney, M.C. Schmidt, Purification and characterization of Snf1 kinase complexes containing a defined Beta subunit composition, J. Biol. Chem. 277 (2002) 50403–50408.
[4] K. Hedbacker, M. Carlson, SNF1/AMPK pathways in yeast, Front. Biosci. 13 (2008) 2408–2420.
[5] S.-P. Hong, F.C. Leiper, A. Woods, D. Carling, M. Carlson, Activation of yeast Snf1 and mammalian AMP-activated protein kinase by upstream kinases, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 8839–8843.
[6] C.M. Sutherland, S.A. Hawley, R.R. McCartney, A. Leech, M.J.R. Stark, M.C. Schmidt, D.G. Hardie, Elm1p is one of three upstream kinases for the *Saccharomyces cerevisiae* SNF1 complex, Curr. Biol. 13 (2003) 1299–1305.
[7] J.L. Celenza, M. Carlson, Mutational analysis of the *Saccharomyces cerevisiae* SNF1 protein kinase and evidence for functional interaction with the SNF4 protein, Mol. Cell. Biol. 9 (1989) 5034–5044.
[8] A. Leech, N. Nath, R.R. McCartney, M.C. Schmidt, Isolation of mutations in the catalytic domain of the snf1 kinase that render its activity independent of the snf4 subunit, Eukaryot. Cell 2 (2003) 265–273.
[9] X. Yang, E.J. Hubbard, M. Carlson, A protein kinase substrate identified by the two-hybrid system, Science 257 (1992) 680–682.
[10] O. Vincent, M. Carlson, Gal83 mediates the interaction of the Snf1 kinase complex with the transcription activator Sip4, EMBO J. 18 (1999) 6672–6681.
[11] O. Vincent, R. Townley, S. Kuchin, M. Carlson, Subcellular localization of the Snf1 kinase is regulated by specific beta subunits and a novel glucose signaling mechanism, Genes Dev. 15 (2001) 1104–1114.
[12] P. Sanz, G.R. Alms, T.A. Haystead, M. Carlson, Regulatory interactions between the Reg1-Glc7 protein phosphatase and the Snf1 protein kinase, Mol. Cell. Biol. 20 (2000) 1321–1328.
[13] A. Ruiz, X. Xu, M. Carlson, Roles of two protein phosphatases, Reg1-Glc7 and Sit4, and glycogen synthesis in regulation of SNF1 protein kinase, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 6349–6354.
[14] D. Castermans, I. Somers, J. Kriel, W. Louwet, S. Wera, M. Versele, V. Janssens, J.M. Thevelein, Glucose-induced posttranslational activation of protein phosphatases PP2A and PP1 in yeast, Cell Res. 22 (2012) 1058–1077.
[15] F. Portillo, J.M. Mulet, R. Serrano, A role for the non-phosphorylated form of yeast Snf1: tolerance to toxic cations and activation of potassium transport, FEBS Lett. 579 (2005) 512–516.
[16] J. Shinoda, Y. Kikuchi, Rod1, an arrestin-related protein, is phosphorylated by Snf1-kinase in *Saccharomyces cerevisiae*, Biochem. Biophys. Res. Commun. 364 (2007) 258–263.
[17] B. Hao, S. Oehlmann, M.E. Sowa, J.W. Harper, N.P. Pavletich, Structure of a Fbw7-Skp1-cyclin E complex: multisite-phosphorylated substrate recognition by SCF ubiquitin ligases, Mol. Cell 26 (2007) 131–143.
[18] S.L. Wade, K. Poorey, S. Bekiranov, D.T. Auble, The Snf1 kinase and proteasome-associated Rad23 regulate UV-responsive gene expression, EMBO J. 28 (2009) 2919–2931.
[19] E.T. Young, C. Zhang, K.M. Shokat, P.K. Parua, K.A. Braun, The AMP-activated protein kinase Snf1 regulates transcription factor binding, RNA polymerase II activity, and mRNA stability of glucose-repressed genes in *Saccharomyces cerevisiae*, J. Biol. Chem. 287 (2012) 29021–29034.
[20] D.G. Chandrashekarappa, R.R. McCartney, M.C. Schmidt, Ligand binding to the AMP-activated protein kinase active site mediates protection of the activation loop from dephosphorylation, J. Biol. Chem. 288 (2013) 89–98.
[21] W.A. Wilson, S.A. Hawley, D.G. Hardie, Glucose repression/derepression in budding yeast: SNF1 protein kinase is activated by phosphorylation under derepressing conditions, and this correlates with a high AMP:ATP ratio, Curr. Biol. 6 (1996) 1426–1434.
[22] F.V. Mayer, R. Heath, E. Underwood, M.J. Sanders, D. Carmena, R.R. McCartney, F.C. Leiper, B. Xiao, C. Jing, P.A. Walker, L.F. Haire, R. Ogrodowicz, S.R. Martin, M.C. Schmidt, S.J. Gamblin, D. Carling, ADP regulates SNF1, the *Saccharomyces cerevisiae* homolog of AMP-activated protein kinase, Cell Metab. 14 (2011) 707–714.
[23] D.G. Chandrashekarappa, R.R. McCartney, M.C. Schmidt, Subunit and domain requirements for adenylate-mediated protection of Snf1 kinase activation loop from dephosphorylation, J. Biol. Chem. 286 (2011) 44532–44541.

[24] C. Dubacq, A. Chevalier, C. Mann, The protein kinase Snf1 is required for tolerance to the ribonucleotide reductase inhibitor hydroxyurea, Mol. Cell. Biol. 24 (2004) 2560–2572.
[25] Y. Liu, X. Xu, M.-H. Kuo, Snf1p regulates Gcn5p transcriptional activity by antagonizing Spt3p, Genetics 184 (2010) 91–105.
[26] S. Pessina, V. Tsiarentsyeva, S. Busnelli, M. Vanoni, L. Alberghina, P. Coccetti, Snf1/AMPK promotes S-phase entrance by controlling CLB5 transcription in budding yeast, Cell Cycle 9 (2010) 2189–2200.
[27] P.T. Spellman, G. Sherlock, M.Q. Zhang, V.R. Iyer, K. Anders, M.B. Eisen, P.O. Brown, D. Botstein, B. Futcher, Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization, Mol. Biol. Cell 9 (1998) 3273–3297.
[28] L.L. Breeden, Periodic transcription: a cycle within a cycle, Curr. Biol. 13 (2003) R31–R38.
[29] C. Wittenberg, R. La Valle, Cell-cycle-regulatory elements and the control of cell differentiation in the budding yeast, BioEssays 25 (2003) 856–867.
[30] L. Alberghina, P. Coccetti, I. Orlandi, Systems biology of the cell cycle of *Saccharomyces cerevisiae*: from network mining to system-level properties, Biotechnol. Adv. 27 (2009) 960–978.
[31] S. Busti, P. Coccetti, L. Alberghina, M. Vanoni, Glucose signaling-mediated coordination of cell growth and cell cycle in *Saccharomyces cerevisiae*, Sensors 10 (2010) 6195–6240.
[32] L. Alberghina, G. Mavelli, G. Drovandi, P. Palumbo, S. Pessina, F. Tripodi, P. Coccetti, M. Vanoni, Cell growth and cell cycle in *Saccharomyces cerevisiae*: basic regulatory design and protein–protein interaction network, Biotechnol. Adv. 30 (2012) 52–72.
[33] M.D. Mendenhall, A.E. Hodge, Regulation of Cdc28 cyclin-dependent protein kinase activity during the cell cycle of the yeast *Saccharomyces cerevisiae*, Microbiol. Mol. Biol. Rev. 62 (1998) 1191–1243.
[34] L. Dirick, T. Moll, H. Auer, K. Nasmyth, A central role for SWI6 in modulating cell cycle start-specific transcription in yeast, Nature 357 (1992) 508–513.
[35] J.F. Partridge, G.E. Mikesell, L.L. Breeden, Cell cycle-dependent transcription of CLN1 involves swi4 binding to MCB-like elements, J. Biol. Chem. 272 (1997) 9071–9077.
[36] I.A. Taylor, P.B. McIntosh, P. Pala, M.K. Treiber, S. Howell, A.N. Lane, S.J. Smerdon, Characterization of the DNA-binding domains from the yeast cell-cycle transcription factors Mbp1 and Swi4, Biochemistry 39 (2000) 3943–3954.
[37] V.R. Iyer, C.E. Horak, C.S. Scafe, D. Botstein, M. Snyder, P.O. Brown, Genomic binding sites of the yeast cell-cycle transcription factors SBF and MBF, Nature 409 (2001) 533–538.
[38] F. Ferrezuelo, N. Colomina, B. Futcher, M. Aldea, The transcriptional network activated by Cln3 cyclin at the G1-to-S transition of the yeast cell cycle, Genome Biol. 11 (2010) R67.
[39] K. Nasmyth, L. Dirick, The role of SWI4 and SWI6 in the activity of G1 cyclins in yeast, Cell 66 (1991) 995–1013.
[40] F.R. Cross, M. Hoek, J.D. McKinney, A.H. Tinkelenberg, Role of Swi4 in cell cycle regulation of CLN2 expression, Mol. Cell. Biol. 14 (1994) 4779–4787.
[41] J.M. Bean, E.D. Siggia, F.R. Cross, High functional overlap between MluI cell-cycle box binding factor and Swi4/6 cell-cycle box binding factor in the G1/S transcriptional program in *Saccharomyces cerevisiae*, Genetics 171 (2005) 49–61.
[42] C. Koch, T. Moll, M. Neuberg, H. Ahorn, K. Nasmyth, A role for the transcription factors Mbp1 and Swi4 in progression from G1 to S phase, Science 261 (1993) 1551–1557.
[43] M.P. Cosma, T. Tanaka, K. Nasmyth, Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter, Cell 97 (1999) 299–311.
[44] M.P. Cosma, S. Panizza, K. Nasmyth, Cdk1 triggers association of RNA polymerase to cell cycle promoters only after recruitment of the mediator by SBF, Mol. Cell 7 (2001) 1213–1220.
[45] L.T. Bhoite, Y. Yu, D.J. Stillman, The Swi5 activator recruits the Mediator complex to the HO promoter without RNA polymerase II, Genes Dev. 15 (2001) 2457–2469.
[46] S. Takahata, Y. Yu, D.J. Stillman, The E2F functional analogue SBF recruits the Rpd3(L) HDAC, via Whi5 and Stb1, and the FACT chromatin reorganizer, to yeast G1 cyclin promoters, EMBO J. 28 (2009) 3378–3389.
[47] S. Takahata, Y. Yu, D.J. Stillman, FACT and Asf1 regulate nucleosome dynamics and coactivator binding at the HO promoter, Mol. Cell 34 (2009) 405–415.
[48] M. Costanzo, J.L. Nishikawa, X. Tang, J.S. Millman, O. Schub, K. Breitkreuz, D. Dewar, I. Rupes, B. Andrews, M. Tyers, CDK activity antagonizes Whi5, an inhibitor of G1/S transcription in yeast, Cell 117 (2004) 899–913.
[49] R.A.M. de Bruin, W.H. McDonald, T.I. Kalashnikova, J. Yates, C. Wittenberg, Cln3 activates G1-specific transcription via phosphorylation of the SBF bound repressor Whi5, Cell 117 (2004) 887–898.
[50] D. Huang, S. Kaluarachchi, D. van Dyk, H. Friesen, R. Sopko, W. Ye, N. Bastajian, J. Moffat, H. Sassi, M. Costanzo, B.J. Andrews, Dual regulation by pairs of cyclin-dependent protein kinases and histone deacetylases controls G1 transcription in budding yeast, PLoS Biol. 7 (2009) e1000188.
[51] S. Hahn, E.T. Young, Transcriptional regulation in *Saccharomyces cerevisiae*: transcription factor regulation and function, mechanisms of initiation, and roles of activators and coactivators, Genetics 189 (2011) 705–736.
[52] T. Formosa, P. Eriksson, J. Wittmeyer, J. Ginn, Y. Yu, D.J. Stillman, Spt16-Pob3 and the HMG protein Nhp6 combine to form the nucleosome-binding factor SPN, EMBO J. 20 (2001) 3506–3517.
[53] M.K. Shirra, R.R. McCartney, C. Zhang, K.M. Shokat, M.C. Schmidt, K.M. Arndt, A chemical genomics study identifies Snf1 as a repressor of GCN4 translation, J. Biol. Chem. 283 (2008) 35889–35898.
[54] P. Coccetti, V. Zinzalla, G. Tedeschi, G.L. Russo, S. Fantinato, O. Marin, L.A. Pinna, M. Vanoni, L. Alberghina, Sic1 is phosphorylated by CK2 on Ser201 in budding yeast cells, Biochem. Biophys. Res. Commun. 346 (2006) 786–793.
[55] K. Baetz, J. Moffat, J. Haynes, M. Chang, B. Andrews, Transcriptional coregulation by the cell integrity mitogen-activated protein kinase Slt2 and the cell cycle regulator Swi4, Mol. Cell. Biol. 21 (2001) 6515–6528.
[56] S. Takahata, Y. Yu, D.J. Stillman, Repressive chromatin affects factor binding at yeast HO (homothallic switching) promoter, J. Biol. Chem. 286 (2011) 34809–34819.
[57] N.F. Lowndes, A.L. Johnson, L. Breeden, L.H. Johnston, SWI6 protein is required for transcription of the periodically expressed DNA synthesis genes in budding yeast, Nature 357 (1992) 505–508.
[58] B.J. Andrews, L.A. Moore, Interaction of the yeast Swi4 and Swi6 cell cycle regulatory proteins in vitro, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 11852–11856.
[59] T. Moll, L. Dirick, H. Auer, J. Bonkovsky, K. Nasmyth, SWI6 is a regulatory subunit of two different cell cycle START-dependent transcription factors in *Saccharomyces cerevisiae*, J. Cell Sci. Suppl. 16 (1992) 87–96.
[60] M. Momcilovic, S.H. Iram, Y. Liu, M. Carlson, Roles of the glycogen-binding domain and Snf4 in glucose inhibition of SNF1 protein kinase, J. Biol. Chem. 283 (2008) 19521–19529.
[61] R.R. McCartney, E.M. Rubenstein, M.C. Schmidt, Snf1 kinase complexes with different beta subunits display stress-dependent preferences for the three Snf1-activating kinases, Curr. Genet. 47 (2005) 335–344.
[62] F. Estruch, M.A. Treitel, X. Yang, M. Carlson, N-terminal mutations modulate yeast SNF1 protein kinase function, Genetics 132 (1992) 639–650.
[63] G. Abate, E. Bastonini, K.A. Braun, L. Verdone, E.T. Young, M. Caserta, Snf1/AMPK regulates Gcn5 occupancy, H3 acetylation and chromatin remodelling at *S. cerevisiae* ADY2 promoter, Biochim. Biophys. Acta 1819 (2012) 419–427.
[64] R. Usaite, M.C. Jewett, A.P. Oliveira, J.R. Yates, L. Olsson, J. Nielsen, Reconstruction of the yeast Snf1 kinase regulatory network reveals its role as a global energy regulator, Mol. Syst. Biol. 5 (2009) 319.
[65] J.-S. Hahn, D.J. Thiele, Activation of the *Saccharomyces cerevisiae* heat shock transcription factor under glucose starvation conditions by Snf1 protein kinase, J. Biol. Chem. 279 (2004) 5169–5176.
[66] I. Mayordomo, F. Estruch, P. Sanz, Convergence of the target of rapamycin and the Snf1 protein kinase pathways in the regulation of the subcellular localization of Msn2, a transcriptional activator of STRE (Stress Response Element)-regulated genes, J. Biol. Chem. 277 (2002) 35650–35656.
[67] V.K. Vyas, S. Kuchin, M. Carlson, Interaction of the repressors Nrg1 and Nrg2 with the Snf1 protein kinase in *Saccharomyces cerevisiae*, Genetics 158 (2001) 563–572.
[68] E.T. Young, N. Kacherovsky, K. Van Riper, Snf1 protein kinase regulates Adr1 binding to chromatin but not transcription activation, J. Biol. Chem. 277 (2002) 38095–38103.
[69] C.J.C. van Oevelen, H.A.A.M. van Teeffelen, F.J. van Werven, H.T.M. Timmers, Snf1p-dependent Spt-Ada-Gcn5-acetyltransferase (SAGA) recruitment and chromatin remodeling activities on the HXT2 and HXT4 promoters, J. Biol. Chem. 281 (2006) 4523–4531.
[70] S.-P. Hong, M. Carlson, Regulation of snf1 protein kinase in response to environmental stress, J. Biol. Chem. 282 (2007) 16838–16845.
[71] V. Cherkasova, H. Qiu, A.G. Hinnebusch, Snf1 promotes phosphorylation of the alpha subunit of eukaryotic translation initiation factor 2 by activating Gcn2 and inhibiting phosphatases Glc7 and Sit4, Mol. Cell. Biol. 30 (2010) 2862–2873.
[72] W.S. Lo, E.R. Gamache, K.W. Henry, D. Yang, L. Pillus, S.L. Berger, Histone H3 phosphorylation can promote TBP recruitment through distinct promoter-specific mechanisms, EMBO J. 24 (2005) 997–1008.
[73] R.G. Jones, D.R. Plas, S. Kubek, M. Buzzai, J. Mu, Y. Xu, M.J. Birnbaum, C.B. Thompson, AMP-activated protein kinase induces a p53-dependent metabolic checkpoint, Mol. Cell 18 (2005) 283–293.
[74] M. Igata, H. Motoshima, K. Tsuruzoe, K. Kojima, T. Matsumura, T. Kondo, T. Taguchi, K. Nakamaru, M. Yano, D. Kukidome, K. Matsumoto, T. Toyonaga, T. Asano, T. Nishikawa, E. Araki, Adenosine monophosphate-activated protein kinase suppresses vascular smooth muscle cell proliferation through the inhibition of cell cycle progression, Circ. Res. 97 (2005) 837–844.
[75] T. Williams, J.E. Brenman, LKB1 and AMPK in cell polarity and division, Trends Cell Biol. 18 (2008) 193–198.
[76] M.R. Banko, J.J. Allen, B.E. Schaffer, E.W. Wilker, P. Tsou, J.L. White, J. Villén, B. Wang, S.R. Kim, K. Sakamoto, S.P. Gygi, L.C. Cantley, M.B. Yaffe, K.M. Shokat, A. Brunet, Chemical genetic screen for AMPKα2 substrates uncovers a network of proteins involved in mitosis, Mol. Cell 44 (2011) 878–892.
[77] A. Vazquez-Martin, S. Cufí, C. Oliveras-Ferraros, J.A. Menendez, Polo-like kinase 1 directs the AMPK-mediated activation of myosin regulatory light chain at the cytokinetic cleavage furrow independently of energy balance, Cell Cycle 11 (2012) 2422–2426.
[78] E. Baena-González, F. Rolland, J.M. Thevelein, J. Sheen, A central integrator of transcription networks in plant stress and energy signalling, Nature 448 (2007) 938–942.