Biochemical and Biophysical Research Communications 423 (2012) 632–637

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Genome-wide location analysis reveals an important overlap between the targets of the yeast transcriptional regulators Rds2 and Adr1

Nitnipa Soontorngun [a,*], Sirilak Baramee [a], Chalinee Tangsombatvichit [a], Piyasuda Thepnok [a], Supapon Cheevadhanarak [b,c], François Robert [d,e], Bernard Turcotte [f]

[a] Division of Biochemical Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, 49 Tianthalay Road, Tha Kham, Bang Khuntian, Bangkok 10150, Thailand
[b] Biotechnology School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, 49 Tianthalay Road, Tha Kham, Bang Khuntian, Bangkok 10150, Thailand
[c] Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bang Khuntian, Bangkok 10150, Thailand
[d] Institut de Recherches Cliniques de Montréal, Montréal, Québec, Canada H2W 1R7
[e] Département de Médecine, Faculté de Médecine Université de Montréal, Montréal, Québec, Canada
[f] Department of Medicine, McGill University Health Centre, Montréal, Québec, Canada H3A 1A1

ARTICLE INFO

Article history:
Received 25 May 2012
Available online 8 June 2012

Keywords:
Saccharomyces cerevisiae
Nonfermentable carbon source
Transcriptional regulation
Zinc cluster protein
Rds2
Adr1

ABSTRACT

Upon glucose depletion, a massive reprogramming of gene expression occurs in the yeast *Saccharomyces cerevisiae* for the use of alternate carbon sources such as the nonfermentable compounds ethanol and glycerol. This process is mediated by the master kinase Snf1 that controls the activity of various targets including the transcriptional regulators Cat8, Sip4 and Adr1. We have recently identified Rds2 as an additional player in this pathway. Here, we have performed genome-wide location analysis of Rds2 in cells grown in the presence of glycerol. We show that Rds2 binds to promoters of genes involved in gluconeogenesis, the glyoxylate shunt, and the TCA cycle as well as some genes encoding mitochondrial components or some involved in the stress response. Interestingly, we also detected Rds2 at the promoters of *SIP4*, *ADR1* and *HAP4* which encodes the limiting subunit of the Hap2/3/4/5 complex, a regulator of respiration. Strikingly, we observed an important overlap between the targets of Rds2 and Adr1. Finally, we provide a model to account for the complex interplay among these transcriptional regulators.

1. Introduction

Glucose is the preferred carbon source for the yeast *Saccaharomyces cerevisiae*. However, if glucose is scarce, this species is able to utilize alternate sugars such as galactose or nonfermentable compounds such as ethanol, lactate or glycerol. A shift from fermentative to nonfermentative growth results in a major reprogramming of gene expression [1,2] and this adaptation is controlled by a network of dedicated transcriptional regulators in the Snf1 signaling pathway [3–5]. Upon glucose depletion, the master kinase Snf1 is activated, resulting in the phosphorylation of various targets including the transcriptional repressor Mig1. Phosphorylated Mig1 is exported out of the nucleus allowing derepression of gluconeogenic genes such as *CAT8* encoding a transcriptional regulator [6]. This allows for induction of *CAT8* expression as well as the target genes of *CAT8*, including *SIP4* that encodes another transcriptional regulator of this pathway [7,8]. Both Cat8 and Sip4 are substrates of the Snf1 kinase and Cat8 phosphorylation was shown to be necessary for its activity [9]. Another important regulator is Adr1 of the C_2H_2 family of zinc finger proteins [5,10]. Adr1 is an activator of a number of genes required for utilization of ethanol, glycerol and fatty acids. For example, Adr1 directly binds and regulates the *GUT1* and *GUT2* genes involved in the very first steps of glycerol utilization [8].

Cat8 and Sip4 belong to the family of zinc cluster proteins which are DNA-binding transcription factors [11]. An additional member of this family is Rds2. We have shown that this factor is a major regulator of gluconeogenesis [12]. Genome-wide location analysis (ChIP-chip) revealed that in glucose-grown cells, Rds2 binds to a limited number of promoters. In contrast, with ethanol as the carbon source, binding of Rds2 is observed at many additional genes including some involved in gluconeogenesis, the TCA cycle and the glyoxylate shunt [12]. Activity of Rds2 is correlated with its phosphorylation by Snf1. As observed for Cat8 and Sip4, Rds2 regulates the expression of key gluconeogenic structural genes such as *PCK1* and *FBP1* encoding PEP carboxykinase and fructose-bisphosphatase, respectively. These enzymes are specific for gluconeogenesis and expression of their genes is tightly regulated according to the carbon source. *PCK1* and *FBP1* expression is strongly repressed in the presence of glucose while they show greatly increased mRNA levels with a nonfermentable carbon

* Corresponding author. Fax: +66 02 452 3479.
E-mail address: nitnipa.soo@kmutt.ac.th (N. Soontorngun).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.05.151

source. In addition, Rds2 is a repressor of the negative gluconeogenic regulators *PFK27* and *VID24* [12]. Rds2 also binds and regulates *HAP4* encoding the limiting subunit of the Hap2/3/4/5 complex involved in controlling expression of respiration genes [12]. In this study, we were interested in determining the targets of Rds2 using glycerol as an alternate carbon source. To this end, ChIP-chip analysis of Rds2 was performed. Overall, our results show that Rds2 has common targets in cells grown in ethanol or glycerol. Importantly, a number of Rds2 targets encode transcriptional regulators such as Adr1, Sip4 and Sut1. In addition, a significant fraction of the promoters bound by Rds2 are also bound by the factor Adr1.

2. Material and methods

2.1. Yeast strains

A previously described strain expressing *RDS2* tagged with a triple HA epitope at its natural chromosomal location was used for ChIP assays and is isogenic to BY4741 (*MAT*a *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) [12]. The double deletion strain Δ*rds2*Δ*cat8* (BY4741 background) was constructed by deleting the *CAT8* open reading frame (ORF) in a Δ*rds2* strain using a PCR approach [13]. The phenotypic analysis of the Δ*rds2* strain was done in the FY73 background (*MATα his3-Δ200 ura3-52*) [14]. The deletion was done by transformation, using the *S. cerevisiae HIS3* marker for selection, as described [15].

2.2. Media and spotting assays

Media were prepared according to Adams [16]. YEP contained 1% yeast extract, 2% peptone supplemented with 2% glucose (YPD) or 2% glycerol, 3% ethanol, 2% acetate, 0.125% oleic or 0.125% linoleic acid. For spotting assays, wild-type and deletion strains were grown overnight at 30 °C in liquid YPD, spun, and resuspended in water. Cells were then serially diluted and spotted on appropriate plates.

2.3. Chromatin immunoprecipitation (ChIP)

ChIP-chip assays were performed as described [17]. Cells from the wild-type (BY4741) and HA-*RDS2* strains were grown in YPD to an approximate OD_{600} of 0.7, washed twice in water, and transferred to YPD or YEP media containing 2% glycerol as a sole carbon source and grown for 3 h. Microarrays used for ChIP-chip (4X 44 K) were obtained from Agilent.

2.4. Peak calling and mapping to genes

"Significantly enriched regions" from the ChIP-chip data were identified as described [18] except that regions smaller than 200 bp pair were extended to 200 bp for downstream analyses. In order to fine point the location of the binding sites within significant regions, the data was interpolated as described [19] and the "maxima" having a log2 ratio >0.8 overlapping with the significantly enriched regions were retained as "significant peaks". The R package HOMER [20] was used in order to map significant peaks to genes. HOMER reports up to 3 genes per peak. The R package HOMER is used to map significant peaks. Genes with promoters (−1000/−100 relative to the start codon) overlapping significant peaks and with 5′ boundaries (defined as the start codon in yeast) closest to peak centers were identified. The output from HOMER was used as a starting point to map significant peaks to genes. The assignments were then manually curated.

Table 1
Oligonucleotides used for standard ChIP and qRT-PCR analysis.

Oligonucleotide	DNA sequence
ChIP analysis	
PCK1	TATCCCACACGATCCACCGG and AACGTACCATTGTCCAACCA
FBP1	ACGCTCTACCAACTGAGCTA and TGATATGTGGGAATACCAGG
LSC2	CATTCATCTCTCCAACTGTA and CGCTCTATAGTCACTGTTAT
SIP4	TATGAGAACTTTTTCCTCAA and CTGCCATATTTCCTCTTGGC
GND1	AGAGAGACCTAAACGTAAGAG and AGCTCAGGAACAATACTGCAG
qRT-PCR	
PCK1	ACAGACTGAATGTCCCCTTC and TATTGACCGGACCCCACCAA
FBP1	TTTGATGTGAATGCCAACTC and TCATCACCTAGAACGTCC
ACS1	GAATGTTTAAATGTCGCCC and TTATCGAATGGCTTAGACC
JEN1	CCCGTGTAGATGTCGTCG and CCTCTTCATCTTCCTCATAC
SIP4	CATCGATTAGATGGCCAAGA and GACCTCTCCTCGACAGTTTA

2.5. Quantitative-RT PCR analysis (qRT-PCR)

For qRT-PCR, wild-type (BY4741) and deletion strains were grown as described above. RNAs were isolated using the hot-phenol extraction method and purified with a RNA clean up kit (Qiagen). cDNAs described synthesis was performed with a Super ScriptIII First-strand synthesis kit from Invitrogen. The qRT-PCR was performed with a Bio-Rad CFX96 using 2X Brilliant SYBR Green QPCR master mix (Kapabiosystem) and gene-specific oligonucleotides (Table 1). The relative quantification of each transcript was calculated by the $2^{-\Delta\Delta CT}$ method [21] using the *ACT1* gene (actin) as an internal control.

3. Results and discussion

3.1. Impaired utilization of multiple non-fermentable carbon sources in cells lacking RDS2 gene

Rds2 is a transcriptional regulator of gluconeogenic genes as shown by ChIP-chip analysis when ethanol is used as a carbon source [12]. We were interested in determining if removal of *RDS2* results in impaired growth with nonfermentable carbon sources. Briefly, wild-type and Δ*rds2* strains were grown overnight in YEP medium containing 2% glucose. Cells were then spun, serially diluted and spotted on YEP plates containing different carbon sources. Consistent with our published results [15], a Δ*rds2* strain (FY73 background) was unable to grow with glycerol as a carbon source (Fig. 1). Similar results were obtained with ethanol or acetate while reduced growth was observed with oleic acid or linoleic acid (Fig. 1). In addition, a Δ*rds2* strain is unable to use lactate as a carbon source [15]. We also observed that the inability of a Δ*rds2* to grow on nonfermentable carbon sources varies according to

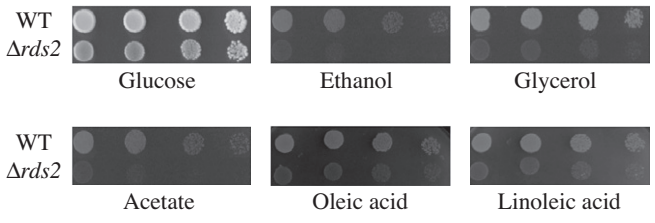

Fig. 1. Impaired growth of the *Δrds2* strain on nonfermentable carbon sources. Growth of a wild-type strain (BY4741) and a *Δrds2* strain were examined on plates containing different carbon sources as indicated on the Figure.

genetic background (data not shown). The explanation for this phenotypic difference is unclear. In the FY73 background, a Δ*rds2* strain has phenotypes characteristic of a respiratory-deficient strain. Thus, the transcriptional regulator Rds2 is required for proper growth on a wide range of non-fermentable carbon sources.

3.2. Rds2 binds to gluconeogenic and glycolytic genes in the presence of glycerol as a carbon source

Then, we focused on gaining insight into the role of Rds2 in the utilization of glycerol as a carbon source. To this end, we performed genome-wide location analysis (ChIP-chip) to uncover genes that are bound by Rds2. ChIP-chip experiments were performed with a strain expressing HA-tagged Rds2 from its natural chromosomal location [12] and microarrays with probes covering almost the entire yeast genome. In glucose-grown cells, Rds2 was bound at a limited number of promoters or intergenic regions (28 targets, $P < 0.05$ with an enrichment >2.5) (See Supplementary Table S1 and S2). Rds2 was mainly found at promoters of genes related to glucogenesis such as *PCK1* (PEP carboxykinase), *MAE1* (mitochondrial malic enzyme), and *PDC1* (pyruvate decarboxylase). When the cells were shifted from glucose to glycerol, 152 promoters or intergenic regions were identified as direct targets of Rds2 ($P < 0.05$ with an enrichment >2.5) (See Supplementary Table S1 and S3). We used these targets to perform a gene ontology search. The most enriched categories are related to oxidation and reduction processes, generation of metabolites and energy, respiration etc. (Table 2). These data are consistent with the role Rds2 plays in nonfermentable carbon metabolism.

With cells grown in the presence of glycerol, binding of Rds2 was observed at promoters of key genes in the gluconeogenic pathway such as *PCK1* and *FBP1* as well as negative regulators of gluconeogenesis such as *PFK27*, *GID8*, *VID24* and *UBC8* (See Supplementary Table S1). These results are consistent with our previous observations when cells were grown with ethanol as a nonfermentable carbon source [12]. ChIP-chip results were confirmed by standard ChIP analysis for *PCK1*, *FBP1* as well as for *LSC2* (encoding a subunit of succinyl-CoA ligase of the TCA cycle) and *SIP4* (Table 1 and Fig. 2). For all of these genes, enrichment was observed with tagged Rds2 as compared to untagged Rds2. No enrichment was observed with *GND1* in standard ChIP (Fig. 2), in agreement with our ChIP-chip results.

Our ChIP-chip analysis revealed binding of Rds2 to promoters of genes encoding some enzymes for glycolysis and related functions (See Supplementary Table S1). For example, Rds2 binds to the promoters of two important hexokinase genes *HXK1* and *HXK2* (Hexokinase enzyme). These two gene products catalyze the conversion of glucose to glucose-6-phosphate. *HXK2* expression is induced by glucose, but repressed by nonfermentable carbons while the opposite pattern is observed for *HXK1* [22]. Binding of Adr1 was also observed at *HXK1* [8]. We also detected binding of Rds2 on the promoters of the *REG1* and *GAC1* genes encoding for two regulatory subunits of Glc7-type1 protein phosphatase that regulates many physiological processes, including glycogen metabolism and glucose repression ([23] and Refs. therein). Reg1 is required for Glc7-dependent deactivation of Snf1 kinase and also plays an important role in glucose-induced degradation of gluconeogenic enzymes via Vid (vacuolar import and degradation) vesicles [24]. Gac1 functions to target Glc7 kinase to the Gsy2 glycogen synthase which is required for glycogen accumulation [25]. Rds2 also binds to promoters of the *HXT2* and *HXT5* gene encoding high- and moderate-affinity glucose transporters, respectively (See Supplementary Table S1). Expression of both genes is repressed by high levels of glucose [4]. Thus, binding of Rds2 on glycolytic and gluconeogenic promoters with cells grown in glycerol suggests possible coordinated regulation of genes in both central pathways during the cellular response to glucose exhaustion and change of available carbon source.

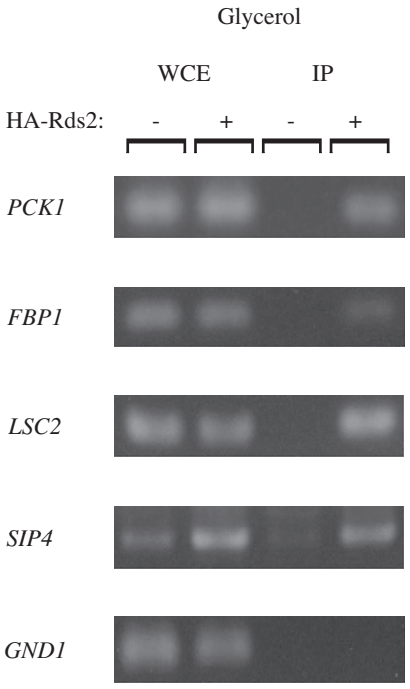

Fig. 2. Confirmation of ChIP-chip results by standard ChIP analysis for some selected genes. Standard ChIP assays were performed with strains expressing untagged (−) or HA-tagged Rds2 (+), grown in rich medium containing glycerol as a sole carbon source. Signals obtained with either input DNA (WCE) or immunoprecipitated DNA (IP) are shown.

Table 2
Gene ontology (GO) enrichment of the Rds2 target genes identified by ChIP-chip analysis. GO terms with a *P*-value smaller than $1X10^{-3}$ are listed.

GO term	*P*-value	Fraction of the query	Fraction of the genome
Oxidation–reduction process	2.2×10^{-7}	37/169	443/6359
Generation/precursors metabolites and energy	3.6×10^{-6}	24/169	222/6359
Coenzyme metabolic process	2.9×10^{-5}	19/169	157/6359
Energy derivation by oxidation of organic compounds	4.4×10^{-5}	19/169	161/6359
Alcohol metabolic process	5.8×10^{-5}	23/169	236/6359
Acetyl-CoA metabolic process	6.7×10^{-5}	10/169	40/6359
Cellular respiration	2.2×10^{-4}	15/169	111/6359
Aerobic respiration	5.2×10^{-4}	13/169	88/6359
Coenzyme catabolite process	8.8×10^{-4}	8/169	30/6359
Tricarboxylic acid cycle	8.8×10^{-4}	8/169	30/6359
Acetyl-CoA catabolic process	8.8×10^{-4}	8/169	30/6359
Alcohol metabolic process	5.8×10^{-5}	23/169	236/6359
Acetyl-CoA metabolic process	6.7×10^{-5}	10/169	40/6359

3.3. Rds2 targets nuclear genes encoding mitochondrial proteins

Growth on a nonfermentable carbon source requires mitochondrial function. Rds2 appears to play an important role in controlling expression of mitochondrial components encoded by the nuclear genome since it binds to many promoters of these genes (See Supplementary Table S1). Targets of Rds2 include *PET9* and *MIR1* encoding mitochondrial carriers for ADP/ATP and phosphate, respectively, as well as genes encoding mitochondrial enzymes (*NDE1*, *PRX1*) or components of enzymatic complexes (e.g. *ATP3*, *ATP5*, *COX4*, *NDE1*, *PRX1*). Additional targets include genes encoding mitochondrial ribosomal proteins (*MRPL35*, *MRPL50*) and genes encoding mitochondrial proteins of unknown function (*AIM17*, *FMP48*, *FAT3*).

3.4. Rds2 binds to promoters of multidrug resistance and stress responsive genes

A diauxic shift is characterized by a general stress response [1]. Rds2 also binds to the promoters of several stress related genes (See Supplementary Table S1). This includes the *ZWF1* gene, required for production of NADPH, a cofactor involved in protection against oxidative stress, as well as *STB5* encoding a regulator of *ZWF1* and other genes of the pentose phosphate pathway [17]. Rds2 is also found at promoters of *GPX1*, *PRX1* and *MTL1* (See Supplementary Table S1 and data not shown). Another prominent group of Rds2 targets is comprised of pleiotropic drug resistance (PDR) genes. A number of studies show that Rds2 modulates drug sensitivity. A Δ*rds2* deletion strain shows increased sensitivity to various toxic compounds such as the antifungal drugs ketoconazole and amphotericin B, the anticancer drug bleomycin [26,27]. Interestingly, binding of Rds2 was detected at promoters of *PDR5*, *PDR16*, *QDR3*, *TPO1* and *AQR1* under glycerol conditions (See Supplementary Table S1). *PDR5* and *PDR15* encode plasma membrane ATP-binding cassette (ABC) transporters which efflux several types of structurally unrelated xenobiotic compounds [28]. *QDR3*, *TPO1* and *AQR1* encode multidrug transporters of the major facilitator superfamily required for resistance to various cytotoxic compounds such as polyamine, quinidine, cisplatin or short-chain monocarboxylic acids ([29] and Refs. therein). Thus, our ChIP-chip data may explain the various phenotypes observed for a strain lacking *RDS2*.

3.5. Contribution of Rds2 and Cat8 to the expression of gluconeogenic genes

We were interested in determining if binding of Rds2 at target promoters is correlated with altered gene expression. To this end, we performed quantitative RT-PCR (qRT-PCR) analysis. We examined the expression levels of the gluconeogenic genes *PCK1*, *FBP1* and others involved in the use of a nonfermentable carbon source *ACS1*, *JEN1* and *SIP4*. qRT-PCR was performed using a wild-type strain and a Δ*rds2* strain. Since Cat8 also binds to the genes listed above, qRT-PCR was also performed using a double deletion strain Δ*rds2*Δ*cat8*. As expected, under glucose conditions, the genes tested were expressed at very low levels while a shift to glycerol greatly increased the expression of these genes (data not shown). Deletion of either *RDS2* or *CAT8* resulted in modest alteration of mRNA levels for the *PCK1*, *FBP1*, *JEN1*, *ACS1* and *SIP4* genes (Fig. 3). In contrast, deletion of both *RDS2* and *CAT8* greatly diminished the expression of *PCK1* and *SIP4* (Fig. 3), while a twofold effect was observed for *JEN1* and *ACS1*, and no significant effect was observed for *FBP1*. These results suggest that there is a redundancy among transcription factors for expression of some genes in this pathway. Overall, our results suggest that both Rds2 and Cat8 positively co-regulate expression of some gluconeogenic genes during the glycerol shift for fine-tuning the control of this complex metabolic process.

3.6. Important overlap between the targets of the yeast transcriptional regulators Rds2 and Adr1

We compared our ChIP-chip analysis of Rds2 with those reported by Tachibana et al. for Cat8 and Adr1 [8]. Rds2 targets that are also bound by Cat8 and Adr1 are indicated in Supplementary Table S3. Out of 152 promoters that are bound by Rds2, 20 (13%) are also recognized by Cat8. Common targets include the gluconeogenic genes *PCK1*, *FBP1*, *MLS1* and *SIP4*. We have previously shown that deletion of *CAT8* results in somewhat reduced binding of Rds2 at *PCK1* and with a more pronounced effect at *FBP1* [12]. However, when looking at Adr1, the overlap between this factor and Rds2 is more important. Indeed, over one third of the Rds2 targets (55/152) are also bound by Adr1. Strikingly, most of the genes bound by both Rds2 and Cat8 are also targets of Adr1 (17 genes out of 21; e.g. *PCK1*, *FBP1*, *HXT5*, *SFC1*). Our results suggest common and specialized roles for these transcription factors. Regarding genes

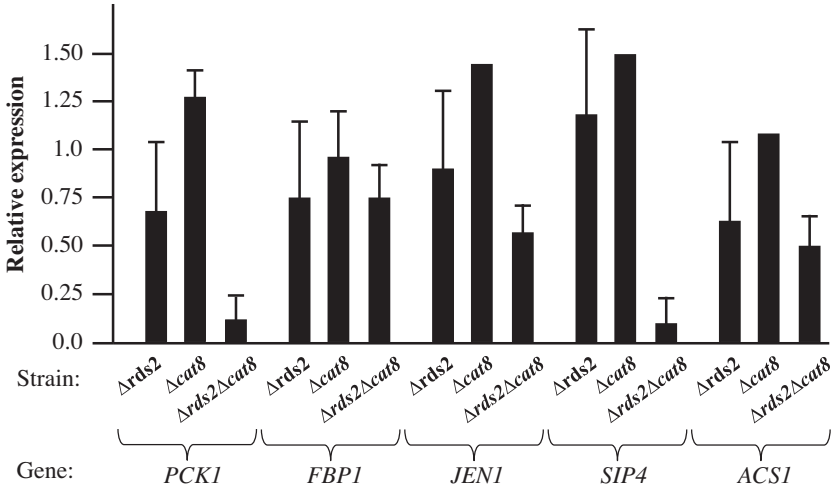

Fig. 3. Derepression of some gluconeogenic genes upon shift to glycerol as carbon source is mediated by both Rds2 and Cat8. Cells were grown in the presence of glycerol as a carbon source and total RNA was isolated for qRT-PCR analysis. RNA levels relative to a wild-type strain are given for genes indicated at the bottom of the figure. Error bars represent standard deviations derived from two representative experiments performed in triplicates.

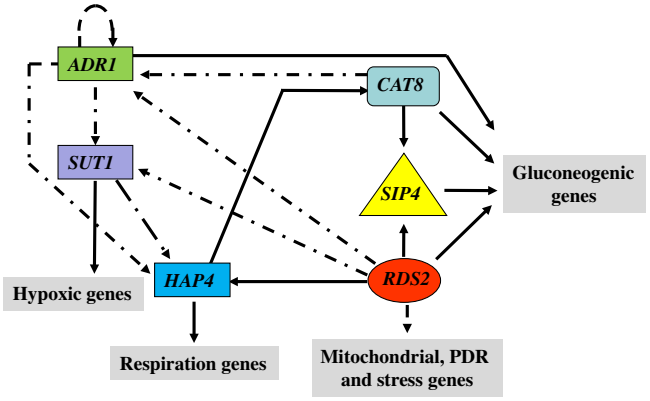

Fig. 4. Model for interplay among transcriptional regulators involved in the utilization of nonfermentable carbon sources. Various transcriptional regulators are shown in the figure. Solid lines with arrows indicate that a given factor binds and regulates the expression of a target. Dashed lines indicate bound targets by a given factor (as inferred from ChIP-chip analysis). Grey boxes represent classes of structural genes that are regulated by a given factor. Data were taken from this study and from those of [8,12,6,32].

specific for glycerol utilization, *STL1* (encoding a glycerol importer) is bound by Adr1 and Cat8 (but not Rds2), while *GUT1* (glycerol kinase) and *GUT2* (glycerol-3-phosphate dehydrogenase) are bound and regulated by Adr1 but, again, not Rds2 [8,30]. Conversely, our ChIP-chip analysis shows that many genes are bound by Rds2 but not Adr1 or Cat8. These genes include negative regulators of gluconeogenesis (*PFK27*, *GID8*, *VID24*, *UBC8*) as well as genes encoding mitochondrial proteins (*RPM2*, *PET9*, *MRPL35*, *MRPL50*, etc.).

Identification of common and individual downstream target genes evidently suggests interplays among these transcriptional factors. We propose a model for this regulatory network of factors involved in the utilization of a nonfermentable carbon source such as glycerol (Fig. 4). Expression of *RDS2* does not vary much according to the carbon source (glucose, glycerol, and ethanol) [2,12]. In contrast, expression of *CAT8*, *SIP4*, *HAP4* and *ADR1* is increased upon a shift to a nonfermentable carbon source while expression of *SUT1* is decreased [1,2]. Sut1, another zinc cluster protein, is involved in sterol uptake under anaerobic conditions [31] but its role under aerobic conditions (if any) is not known. Rds2 binds to the promoter of *HAP4* under both glycerol and ethanol supplemented conditions (See supplementary Table S1 and [12]). We have previously shown that deletion of *RDS2* results in decreased levels of *HAP4*. Thus, upon activation of Rds2 by the Snf1 kinase, Rds2 can positively control the expression of *HAP4*. There is evidence that Hap4 is a positive regulator of *CAT8* since deletion of *HAP2* (encoding a subunit of the Hap2/3/4/5 complex) results in decreased activity of a *CAT8-lacZ* reporter [6]. Rds2 and Cat8 bind to and regulate the expression of *SIP4* (Fig. 2). Regulation is further complicated by the fact that Cat8 and Rds2 bind to the *ADR1* promoter. Adr1 also binds to its own promoter suggesting a positive autoregulatory loop. Finally, *SUT1* appears to be another member of this network. Strikingly, both Adr1 and Rds2 bind to the promoter of the *SUT1* gene while Sut1 is found at *HAP4*. These observations suggest a complex interplay among these factors. Clearly, additional experiments are required to better characterize the connections of this network and to better understand its dynamics.

Acknowledgments

We are thankful to Drs. Mark Featherstone, PhuaSze Calista (Nanyang Technological University), Khanok Ratanakhanokchai (KMUTT) and Wananit Wimuttisuk (NSTDA) for support. We thank Xiao Guo for construction of a yeast strain. Critical review of the manuscript by Dr. Bennett is acknowledged. This work was supported by grants from Thailand Research Fund and Office of the Higher Education Commission (grant #MRG5280184) and National Research Council of Thailand to NS, by a grant from the Natural Sciences and Engineering Research Council to BT (grant #184053-09) and by a grant from the Canadian Institutes of Health Research to FR (grant #82891).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.151.

References

[1] J.L. DeRisi, V.R. Iyer, P.O. Brown, Exploring the metabolic and genetic control of gene expression on a genomic scale, Science 278 (1997) 680–686.
[2] G.G. Roberts, A.P. Hudson, Transcriptome profiling of *Saccharomyces cerevisiae* during a transition from fermentative to glycerol-based respiratory growth reveals extensive metabolic and structural remodeling, Mol. Genet. Genomics 276 (2006) 170–186.
[3] H.-J. Schuller, Transcriptional control of nonfermentative metabolism in the yeast *Saccharomyces cerevisiae*, Curr. Genet. 43 (2003) 139–160.
[4] S. Zaman, S.I. Lippman, X. Zhao, J.R. Broach, How *Saccharomyces* responds to nutrients, Annu. Rev. Genet. 42 (2008) 27–81.
[5] B. Turcotte, X.B. Liang, F. Robert, N. Soontorngun, Transcriptional regulation of nonfermentable carbon utilization in budding yeast, FEMS Yeast Res. 10 (2010) 2–13.
[6] A. Rahner, A. Scholer, E. Martens, B. Gollwitzer, H.J. Schuller, Dual influence of the yeast Cat1p (Snf1p) protein kinase on carbon source-dependent transcriptional activation of gluconeogenic genes by the regulatory gene *CAT8*, Nucleic Acids Res. 24 (1996) 2331–2337.
[7] V. Haurie, M. Perrot, T. Mini, P. Jeno, F. Sagliocco, H. Boucherie, The transcriptional activator Cat8p provides a major contribution to the reprogramming of carbon metabolism during the diauxic shift in *Saccharomyces cerevisiae*, J. Biol. Chem. 276 (2001) 76–85.
[8] C. Tachibana, J.Y. Yoo, J.-B. Tagne, N. Kacherovsky, T.I. Lee, E.T. Young, Combined global localization analysis and transcriptome data identify genes that are directly coregulated by Adr1 and Cat8, Mol. Cell. Biol. 25 (2005) 2138–2146.
[9] G. Charbon, K.D. Breunig, R. Wattiez, J. Vandenhaute, I. Noel-Georis, Key role of Ser562/661 in Snf1-dependent regulation of Cat8p in *Saccharomyces cerevisiae* and *Kluyveromyces lactis*, Mol. Cell. Biol. 24 (2004) 4083–4091.
[10] 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.
[11] S. MacPherson, M. Larochelle, B. Turcotte, A fungal family of transcriptional regulators: the zinc cluster proteins, Microbiol. Mol. Biol. Rev. 70 (2006) 583–604.
[12] N. Soontorngun, M. Larochelle, S. Drouin, F. Robert, B. Turcotte, Regulation of gluconeogenesis in *Saccharomyces cerevisiae* is mediated by activator and repressor function of Rds2, Mol. Cell. Biol. 27 (2007) 7895–7905.
[13] A. Baudin, O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute, C. Cullin, A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*, Nucleic Acids Res. 21 (1993) 3329–3330.
[14] F. Winston, C. Dollard, S.L. Ricupero-Hovasse, Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C, Yeast 11 (1995) 53–55.
[15] B. Akache, K. Wu, B. Turcotte, Phenotypic analysis of genes encoding yeast zinc cluster proteins, Nucleic Acids Res. 29 (2001) 2181–2190.
[16] A. Adams, D.E. Gottschling, T. Stearns, Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1997.
[17] M. Larochelle, S. Drouin, F. Robert, B. Turcotte, Oxidative stress-activated zinc cluster protein Stb5 has dual activator/repressor functions required for pentose phosphate pathway regulation and NADPH production, Mol. Cell. Biol. 26 (2006) 6690–6701.
[18] L.A. Boyer, T.I. Lee, M.F. Cole, S.E. Johnstone, S.S. Levine, J.P. Zucker, M.G. Guenther, R.M. Kumar, H.L. Murray, R.G. Jenner, D.K. Gifford, D.A. Melton, R. Jaenisch, R.A. Young, Core transcriptional regulatory circuitry in human embryonic stem cells, Cell 122 (2005) 947–956.
[19] B. Guillemette, A.R. Bataille, N. Gevry, M. Adam, M. Blanchette, F. Robert, L. Gaudreau, Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning, PLoS Biol. 3 (2005) e384.
[20] S. Heinz, C. Benner, N. Spann, E. Bertolino, Y.C. Lin, P. Laslo, J.X. Cheng, C. Murre, H. Singh, C.K. Glass, Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities, Mol. Cell 38 (2010) 576–589.
[21] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods 25 (2001) 402–408.

[22] J.A. Barnett, K.-D. Entian, A history of research on yeasts 9: regulation of sugar metabolism, Yeast 22 (2005) 835–894.
[23] Y.S. Tan, P.A. Morcos, J.F. Cannon, Pho85 phosphorylates the Glc7 protein phosphatase regulator Glc8 in vivo, J. Biol. Chem. 278 (2003) 147–153.
[24] D.Y. Cui, C.R. Brown, H.L. Chiang, The type 1 phosphatase Reg1p-Glc7p is required for the glucose-induced degradation of fructose-1,6-bisphosphatase in the vacuole, J. Biol. Chem. 279 (2004) 9713–9724.
[25] D. Huang, J. Moffat, W.A. Wilson, L. Moore, C. Cheng, P.J. Roach, B. Andrews, Cyclin partners determine Pho85 protein kinase substrate specificity in vitro and in vivo: control of glycogen biosynthesis by Pcl8 and Pcl10, Mol. Cell. Biol. 18 (1998) 3289–3299.
[26] I. Moreno, N. Tutrone, R. Sentandreu, E. Valentin, Saccharomyces cerevisiae Rds2 transcription factor involvement in cell wall composition and architecture, Int. Microbiol. 11 (2008) 57–63.
[27] B. Akache, B. Turcotte, New regulators of drug sensitivity in the family of yeast zinc cluster proteins, J. Biol. Chem. 277 (2002) 21254–21260.
[28] H.B. van den Hazel, H. Pichler, M.A. do Valle Matta, E. Leitner, A. Goffeau, G. Daum, *PDR16* and PDR17, two homologous genes of *Saccharomyces cerevisiae*, affect lipid biosynthesis and resistance to multiple drugs, J. Biol. Chem. 274 (1999) 1934–1941.
[29] Y. Gbelska, J.J. Krijger, K.D. Breunig, Evolution of gene families: the multidrug resistance transporter genes in five related yeast species, FEMS Yeast Res. 6 (2006) 345–355.
[30] E.T. Young, K.M. Dombek, C. Tachibana, T. Ideker, Multiple pathways are co-regulated by the protein kinase Snf1 and the transcription factors Adr1 and Cat8, J. Biol. Chem. 278 (2003) 26146–26158.
[31] F. Ness, S. Bourot, M. Regnacq, R. Spagnoli, T. Berges, F. Karst, *SUT1* is a putative Zn[II]2Cys6-transcription factor whose upregulation enhances both sterol uptake and synthesis in aerobically growing *Saccharomyces cerevisiae* cells, Eur. J. Biochem. 268 (2001) 1585–1595.
[32] C.T. Harbison, D.B. Gordon, T.I. Lee, N.J. Rinaldi, K.D. Macisaac, T.W. Danford, N.M. Hannett, J.B. Tagne, D.B. Reynolds, J. Yoo, E.G. Jennings, J. Zeitlinger, D.K. Pokholok, M. Kellis, P.A. Rolfe, K.T. Takusagawa, E.S. Lander, D.K. Gifford, E. Fraenkel, R.A. Young, Transcriptional regulatory code of a eukaryotic genome, Nature 431 (2004) 99–104.