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Transcriptional regulation of nonfermentable carbon utilization in budding yeast

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Abstract

Saccharomyces cerevisiae preferentially uses glucose as a carbon source, but following its depletion, it can utilize a wide variety of other carbons including nonfermentable compounds such as ethanol. A shift to a nonfermentable carbon source results in massive reprogramming of gene expression including genes involved in gluconeogenesis, the glyoxylate cycle, and the tricarboxylic acid cycle. This review is aimed at describing the recent progress made toward understanding the mechanism of transcriptional regulation of genes responsible for utilization of nonfermentable carbon sources. A central player for the use of nonfermentable carbons is the Snf1 kinase, which becomes activated under low glucose levels. Snf1 phosphorylates various targets including the transcriptional repressor Mig1, resulting in its inactivation allowing derepression of gene expression. For example, the expression of *CAT8*, encoding a member of the zinc cluster family of transcriptional regulators, is then no longer repressed by Mig1. Cat8 becomes activated through phosphorylation by Snf1, allowing upregulation of the zinc cluster gene *SIP4*. These regulators control the expression of various genes including those involved in gluconeogenesis. Recent data show that another zinc cluster protein, Rds2, plays a key role in regulating genes involved in gluconeogenesis and the glyoxylate pathway. Finally, the role of additional regulators such as Adr1, Ert1, Oaf1, and Pip2 is also discussed.

Keywords

Saccharomyces cerevisiae; nonfermentable carbon; gluconeogenesis; transcriptional regulator; zinc cluster protein

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Introduction

As observed in many unicellular organisms, the budding yeast *Saccharomyces cerevisiae* preferentially uses glucose over other carbon sources as it can directly enter the glycolytic pathway. However, when glucose is unavailable, alternative carbon sources are used for the production of metabolic energy and cellular biomass. Budding yeast is able to utilize a wide variety of different carbons; for example, other alternative sugars such as galactose, sucrose, maltose, and melbiose as well as nonsugar carbons such as ethanol, lactate, glycerol, acetate, or oleate may be used. The enzymatic pathways required for the specific utilization of these carbon compounds are very well characterized. Quite often, enzymes needed for a specific pathway are produced only when required. This regulation is mainly (but not exclusively) exerted at the transcriptional level. A classical example is the galactose-induced expression of genes required for catabolism of this sugar by the transcriptional activator Gal4 (Lohr *et al.*, 1995). Various groups have reviewed the utilization of alternate carbon sources in *S. cerevisiae* (Gancedo, 1998; Carlson, 1999; Schüller, 2003; Barnett & Entian, 2005; Gurvitz & Rottensteiner, 2006b; Zaman *et al.*, 2008). This current review is aimed at highlighting the recent progress made toward better understanding the transcriptional regulation of genes involved in the use of nonfermentable carbon sources.

A shift from one carbon source to another is referred to as a diauxic shift, where exhaustion of a preferred carbon source will be followed by considerably reduced growth leading to adaptation for using an alternate supply for carbon. The name diauxic was first described in *Escherichia coli* for adaptation to the use of lactose upon glucose exhaustion. Another classical example of a diauxic shift is provided by yeast with a shift from a fermentative to a nonfermentative mode of growth. During this transition, a massive reprogramming of expression occurs for genes in various pathways such as carbon metabolism, protein synthesis, and carbohydrate storage (DeRisi *et al.*, 1997). Fitness experiments with pooled deletion strains showed that over 600 genes are required for optimal growth with nonfermentable carbons such as ethanol (Steinmetz *et al.*, 2002). The upregulation of gluconeogenic gene expression is indispensable for the production of glucose-6-phosphate, which is critical for cell growth. For instance, glucose-6-phosphate is required for nucleotide metabolism, glycosylation, cell wall biosynthesis, and storage of carbohydrates (Barnett & Entian, 2005). The expression of gluconeogenic genes is coregulated with the expression of many respiratory genes, as respiration is necessary in order to obtain energy by oxidative phosphorylation during gluconeogenic processes; as a result, respiratory-deficient mutants are unable to grow on the nonfermentable carbon sources (Hampsey, 1997). Biosynthesis of mitochondrial proteins depends on the presence of oxygen and heme and the availability of a carbon source (Schüller, 2003). For example, the expression of mitochondrial genes is increased in the presence of glycerol as compared with glucose (Roberts & Hudson, 2006).

Metabolism of nonfermentable carbons

Metabolism of glycerol

Yeast cells use glycerol as a carbon source as well as for osmoregulation (Hohmann, 2002). Glycerol uptake is mediated by the symporter Stl1 (sugar transporter-like protein) (Ferreira *et al.*, 2005) (Fig. 1). Following its uptake, glycerol is converted to glycerol-3-phosphate by

the cytoplasmic kinase Gut1 before entering the mitochondria. The mitochondrial FAD-dependent glycerol-3-phosphate dehydrogenase, encoded by the *GUT2* gene, is responsible for the conversion to dihydroacetone phosphate, which can enter the glycolytic or the gluconeogenic pathway. Both *GUT1* and *GUT2* are expressed with cells grown in the presence of glycerol or ethanol while these genes are repressed in the presence of glucose (Pavlik *et al.*, 1993).

Metabolism of lactate, ethanol, and acetate

In contrast to glycerol, lactate is taken up in the cells through a specific permease called Jen1 that also transports pyruvate (Casal *et al.*, 1999; reviewed in Casal *et al.*, 2008). *JEN1* expression is repressed in the presence of glucose and is induced by lactate. D-Lactate and L-lactate are metabolized to pyruvate by two distinct mitochondrial lactate cytochrome *c* oxidoreductases, encoded by the *DLD1* and *CYB2* genes, respectively (Lodi & Ferrero, 1999). Unlike glycerol or lactate, ethanol and acetate are thought to enter the cells by passive diffusion, although an acetate carrier has been identified (Casal *et al.*, 1996) (Fig. 1). Ethanol is also produced routinely in the cell as a consequence of alcoholic fermentation. Following its uptake, ethanol is metabolized to acetaldehyde by alcohol dehydrogenase (encoded by *ADH2*) and to acetate by aldehyde dehydrogenase (*ALD6*). Acetate is then transformed to acetyl-CoA by acetyl-CoA synthetase (*ACS1*).

Gluconeogenesis

Glycolysis and gluconeogenesis are two opposite pathways for glucose metabolism and multiple levels of regulation insure that only one pathway is active at a time. For example, the gluconeogenic enzymes fructose-1,6-bisphosphatase (*FBP1*), malate dehydrogenase (*MDH2*), and phosphoenolpyruvate carboxykinase (*PCK1*) are subject to degradation in the presence of glucose (Hung *et al.*, 2004; Santt *et al.*, 2008). Interestingly, the enzymatic activity of Pck1 requires acetylation at lysine 514 by the NuA4 acetyltransferase complex. This post-translational modification is essential for the growth of yeast cells on nonfermentable carbon sources (Lin *et al.*, 2009). Allosteric control of enzymatic activity is also observed (Heinisch *et al.*, 1996). Moreover, mRNA stability of some gluconeogenic genes is increased in the presence of a non-fermentable carbon source (Lombardo *et al.*, 1992; Mercado *et al.*, 1994; Andrade *et al.*, 2005). Finally, another important mechanism of regulation is exerted at the transcriptional level. For instance, the expression of the gluconeogenic genes *PCK1* and *FBP1* as well as genes encoding glyoxylate enzymes *ICL1* (isocitrate lyase) and *MLS1* (malate synthase) is considerably upregulated during glucose depletion.

A number of enzymes are common to both glycolytic and gluconeogenic pathways while three enzymes are specific to gluconeogenesis, as described hereafter. Oxaloacetate is produced from pyruvate by pyruvate carboxylase encoded by the *PYC1* and *PYC2* genes. Oxaloacetate is then converted to phosphoenolpyruvate by the *PCK1* gene product. A series of reactions allow the production of fructose-1,6-bisphosphate. The gluconeogenic enzyme fructose-1,6-bisphosphatase converts this compound to fructose-6-phosphate, which then yields glucose-6-phosphate by a reaction performed by phosphoglucose isomerase (*PGI1*).

Metabolism of oleic acid

The presence of oleate as a sole carbon source results in the upregulation of genes encoding enzymes for fatty acid β-oxidation and proteins involved in the enlargement of peroxisomes (reviewed in Hiltunen *et al.*, 2003; Gurvitz & Rottensteiner, 2006a). There is evidence that the transporter Fat1 and the acyl-CoA synthetases Faa1 and Faa4 mediate active intracellular import (and activation) of fatty acids (Black & DiRusso, 2007). A heterodimer of the ATP-binding cassette transporters Pxa1 and Pxa2 is responsible for transport of activated fatty acids into the peroxisome, where β-oxidation takes place (Hiltunen *et al.*, 2003). Enzymes involved in fatty acid oxidation include Fox1/Pox1 (a fatty-acyl coenzyme A oxidase), Fox2 (a protein with dual activity: 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase), and Pot1/Fox3 (a 3-ketoacyl-CoA thiolase).

Transcriptional regulators: the zinc cluster proteins

A number of transcriptional regulators implicated in the use of alternate carbon sources have been identified and are listed in Table 1. Many of them belong to the Gal4 family and form a subclass of zinc finger proteins called zinc binuclear cluster or zinc cluster proteins (Vallee *et al.*, 1991). Zinc cluster proteins form one of the largest families of transcriptional regulators in the yeast *S. cerevisiae*, consisting of over 50 members (MacPherson *et al.*, 2006). They are characterized by the presence of a well-conserved and fungal-specific zinc cluster motif, $CysX_2CysX_6$ $CysX_{5–12}CysX_2CysX_{6–8}Cys$, located in the DNA-binding domain (Todd & Andrianopoulos, 1997; MacPherson *et al.*, 2006). The proper folding of this domain is co-ordinated through the binding of the conserved cysteine residues to two zinc atoms. Mutation or deletion of these cysteines, or the absence of zinc, results in the loss of DNA-binding activity (Bai & Kohlhaw, 1991). The zinc cluster motif makes contact with three base pairs, usually CGG triplets, in the major groove of the DNA (Marmorstein *et al.*, 1992; Marmorstein & Harrison, 1994). Altering the spacing between the triplets generates binding sites for different zinc cluster proteins. Variation in the relative orientation of the CGG triplets [inverted (CGG Nx CCG), direct (CGG Nx CGG), or everted (CCG Nx CGG) repeats] further increases the repertoire of binding sites for these regulators (Mac-Pherson *et al.*, 2006). Quite often, zinc cluster proteins bind to DNA as homo- or heterodimers although monomeric binding has also been described (MacPherson *et al.*, 2006). Zinc cluster proteins can act as transcriptional activators or repressors and some of them have been shown to perform both functions (Larochelle *et al.*, 2006; MacPherson *et al.*, 2006; Soontorngun *et al.*, 2007).

A number of zinc cluster regulators play central roles in co-ordinating gene expression during adaptation to different carbon sources. For example, Gal4 and its control of *GAL* structural genes for galactose catabolism is a classic example of eukaryotic transcriptional regulation (Lohr *et al.*, 1995; Traven *et al.*, 2006). Three other zinc cluster proteins, Mal13, Mal3R, and Mal63, are involved in the control of maltose metabolic genes in some yeast strains (Needleman, 1991). Other zinc cluster proteins described below are involved in the use of nonfermentable carbons.

Role of the zinc cluster proteins Cat8 and Sip4

Schöler & Schüller (1994) previously reported the presence of a carbon source-responsive element (CSRE) in the promoter of *ICL1*-encoding isocitrate lyase, a key enzyme of the glyoxylate cycle. They showed that the CSRE is an element necessary for *ICL1* derepression in the absence of glucose. Additionally, it was shown that the CSRE alone allows for transcription on a heterologous minimal promoter in a carbon source-dependent manner. A number of other genes also contain CSREs in their promoters [consensus sequence: YCCRTTNRNCGG (Roth *et al.*, 2004)]: *FBP1*, *PCK1*, *MLS1*, *ACS1*, *MDH2* (malate dehydrogenase), *SFC1* (succinate/fumarate transporter), *CAT2* (carnitine acetyltransferase), *IDP2* (NADP-dependent isocitrate dehydrogenase), and *JEN1* (Schüller, 2003). Activation of genes containing CSREs is mediated, among others, by the zinc cluster proteins Cat8 (CATabolite repression) and Sip4, which was isolated as an Snf1-interacting protein (Hedges *et al.*, 1995; Lesage *et al.*, 1996; Rahner *et al.*, 1996; Vincent & Carlson, 1998). Snf1 is a central serine–threonine kinase in the signaling pathway for glucose-mediated repression.

Other studies showed that both Cat8 and Sip4 bind to CSREs in the promoter of gluconeogenic genes *in vitro* (Vincent & Carlson, 1998; Rahner *et al.*, 1999). Although these two activators are involved in gluconeogenesis, their relative contribution via the CSRE is different. A substantial reduction in the expression of CSRE-dependent genes was shown in the absence of Cat8, while removal of Sip4 accounted for only a minor reduction in gene activation (Hiesinger *et al.*, 2001). Additionally, cells lacking Cat8, but not Sip4, are unable to grow on nonfermentable carbon sources (Hedges *et al.*, 1995; Rahner *et al.*, 1996).

The expression of the transcriptional regulator Cat8 is under the control of the carbon source (Hedges *et al.*, 1995; Randez-Gil *et al.*, 1997). In the presence of glucose, *CAT8* expression is repressed by Mig1 (a Cys_2His_2 zinc finger protein), possibly by direct binding of this regulator to the *CAT8* promoter (Hedges *et al.*, 1995; Rahner *et al.*, 1996). A related regulatory mechanism applies to another CSRE-binding protein, Sip4. Derepression of CSRE-containing genes is abolished in a Δ*cat8*Δ*sip4* deletion mutant, suggesting their role as sole activators specific for the CSRE motif (Roth *et al.*, 2004). However, evidence suggests that they may utilize different CSRE variants and that Sip4 actually recognizes a narrower range of binding sites as compared with Cat8 (Roth *et al.*, 2004). Importantly, Cat8 is an activator of *SIP4* transcription and, therefore, indirectly of Sip4 target genes (Haurie *et al.*, 2001; Tachibana *et al.*, 2005). A CSRE-like element is found on the *SIP4* promoter, which may explain the carbon source-dependent activation of *SIP4* expression (Vincent & Carlson, 1998). In agreement with this hypothesis, a microarray study showed that the transcription of *SIP4* is induced approximately ninefold during a diauxic shift (DeRisi *et al.*, 1997). Moreover, deletion of *CAT8* results in a reduction of *SIP4* mRNA, further arguing for a crosstalk between these two genes (Haurie *et al.*, 2001).

Role of the zinc cluster protein Rds2

Recently, another zinc cluster protein was described as being important for regulating gluconeogenesis (Soontorngun *et al.*, 2007). A number of phenotypes are associated with a deletion of the ORF of *YPL133C* including sensitivity to calcofluor white and the antifungal

drug ketoconazole, and it was named *RDS2* (for regulator of drug sensitivity) (Akache *et al.*, 2001; Akache & Turcotte, 2002). Depending on the strain background, impaired growth on glycerol or lactate is also observed with a partial deletion of *RDS2* (Akache *et al.*, 2001). ChIP-chip, a technique that relies on chromatin immunoprecipitation (ChIP) and microarray (chip), was used to determine the genome-wide localization of Rds2. Results showed that this factor binds to a limited number of promoters with cells grown in the presence of glucose while it binds to many additional genes when ethanol is used as a carbon source. Strikingly, the genes bound by Rds2 are involved in gluconeogenesis (e.g. *PCK1*) and related pathways such as the glyoxylate shunt and the tricarboxylic acid cycle. Importantly, it was shown that Rds2 acts as a transcriptional activator of gluconeogenic genes while it is a repressor of the negative regulators of gluconeogenesis. Genes under the positive regulation of Rds2 include *PCK1*, *FBP1*, and *LSC2*. In the absence of *RDS2*, the expression of *GID8* (glucose-induced degradation) is increased with cells grown in the presence of ethanol. Gid8 is a part of a complex involved in the degradation of Fbp1 and Pck1 under glucose conditions (Regelmann *et al.*, 2003; Santt *et al.*, 2008). These results suggest that, following a shift from glucose to ethanol, the expression of *GID8* is repressed to prevent degradation of gluconeogenic enzymes by the Gid complex. Similarly, under ethanol conditions, Rds2 is a repressor of the *PFK27* gene. Pfk27 catalyzes the production of fructose-2,6-bisphosphate, an allosteric activator of the glycolytic enzyme phosphofructokinase (*PFK1*,*2*) and a repressor of Fbp1 (Fig. 1) (Noda *et al.*, 1984; Heinisch *et al.*, 1996). Thus, Rds2 has activator and repressor functions that contribute to the selective activation of gluconeogenesis over glycolysis.

The importance of *RDS2* in controlling genes involved in ethanol utilization is further exemplified by the fact that it binds and upregulates the expression of *HAP4*. The Hap2/3/4/5 complex controls the expression of respiration genes via an activating subunit encoded by *HAP4*, the only subunit whose expression is regulated by a carbon source (Forsburg & Guarente, 1989; DeRisi *et al.*, 1997). This effect may be mediated by a functional CSRE present in the *HAP4* promoter (Brons *et al.*, 2002). Moreover, Rds2 binding is also detected at the *OPI1* promoter, encoding a negative regulator of the phospholipid biosynthetic pathway. The connection between phospholipids and Rds2 may not be obvious. However, the *GUT1* and the *GUT2* genes, involved in glycerol utilization, were shown to be negatively regulated by the repressor Opi1 (Grauslund *et al.*, 1999; Grauslund & Ronnow, 2000). Deletion of *OPI1* allows derepression of *GUT1*, as assayed in glucose. Thus, Rds2 may positively regulate the expression of *GUT1* and *GUT2* indirectly by repressing *OPI1* expression in the presence of nonfermen-table carbons (but not in the presence of glucose). Rds2 also binds to the regulatory gene *SIP4*, raising the possibility that both Cat8 and Rds2 control *SIP4* expression. As observed for Cat8 and Sip4, the purified DNA-binding domain of Rds2 binds *in vitro* to CSREs, and mutations diminishing Cat8 binding also affect the binding of Rds2 (Soontorngun *et al.*, 2007). In summary, Rds2 is a newly characterized transcriptional regulator playing a central role in the regulation of gluconeogenesis in yeast.

Role of the zinc finger protein Adr1

Adr1 is a transcription factor of the Cys_2His_2 class of zinc finger that binds DNA as a monomer (Thukral *et al.*, 1991; Cheng *et al.*, 1994). Adr1 is involved in regulating genes for

utilization of ethanol, glycerol, and lactate (Simon *et al.*, 1991; Young *et al.*, 2003). In fact, the expression of over 100 genes is dependent on Adr1, as shown by microarray analysis (Young *et al.*, 2003). For example, Adr1 regulates the expression of over 30 glucose-repressed genes such as *ADH2*, encoding an alcohol dehydrogenase acting at the first step of ethanol utilization (Fig. 1). Other genes regulated by Adr1 include *ALD4*, *ACS1*, *GUT1*, and *FOX2*. Adr1 and Cat8 coregulate some genes such as *JEN1*, although expression profiling and ChIP-chip data indicate that only a handful (14) of overlapping gene targets is shared between them (Young *et al.*, 2003; Tachibana *et al.*, 2005). Similarly, a comparison of the ChIP-chip data obtained with Cat8 (under low glucose conditions) and Rds2 (ethanol) shows that these factors have only a limited number of common targets that include *PCK1*, *MDH2*, and *SFC1* (Fig. 2) (Soontorngun *et al.*, 2007).

The zinc cluster protein Ert1

A recent study identified the zinc cluster genes *AcuM* and *AcuK* as being involved in regulating the transcription of gluconeogenic genes in the filamentous fungi *Aspergillus nidulans* (Hynes *et al.*, 2007). *AcuM* appears to be a homologue of Rds2 while AcuK shows a strong similarity to the zinc cluster protein Ybr239c (alias Ert1) in *S. cerevisiae*. Interestingly, a large-scale two-hybrid study in budding yeast suggested a physical interaction between Rds2 and Ert1 (Ito *et al.*, 2001). To learn more about the role of Ert1, ChIP analysis was performed with this factor and binding was observed at the *PCK1* promoter (X.B. Liang & B. Turcotte, unpublished data). Moreover, deletion of *ERT1* results in a slight decrease of the expression of *PCK1* (X.B. Liang & B. Turcotte, unpublished data). The exact role of this zinc cluster protein remains to be defined. Taken together, the results show that at least four zinc cluster proteins (Cat8, Sip4, Rds2, and Ert1) can bind to the *PCK1* promoter. Clearly, a complex regulation is exerted at this gene encoding a key component of gluconeogenesis. The specific role of these factors and their interplay at *PCK1* (and other genes) remains to be defined more precisely.

The zinc cluster protein Gsm1

Other studies suggest that another zinc cluster protein is also implicated in the use of nonfermentable carbon sources. Indeed, an expression profiling study showed that mRNA levels of the zinc cluster gene *GSM1* (glucose starvation modulator) are increased 12 times in the presence of glycerol or ethanol, as compared with glucose (Roberts & Hudson, 2006). Moreover, ChIP-chip experiments show that this protein binds, for example, to the *HAP4* and *IDP2* promoters (van Bakel *et al.*, 2008). Gsm1 regulates the expression of the gluconeogenic genes *PCK1* and *FBP1* (W.G. Bao & M. Bolotin-Fukuhara, pers. commun.). Interestingly, the expression of *GSM1* is decreased in cells lacking *HAP2* or *HAP4* (Buschlen *et al.*, 2003). These results suggest an interplay between *HAP4* and *GSM1*.

It remains to be seen whether additional factors may be involved in the use of nonfermentable carbons. Its transcriptional regulation involves more regulatory factors than initially anticipated. The roles of specific transcriptional regulators may also differ according to the nonfermentable carbon. For example, ChIP-chip analysis of Rds2 under lactate shows that its targets differ from those identified under ethanol conditions (N. Soontorngun & B.

Turcotte, unpublished data). As stated above, Rds2 and Ert1 interact with each other in a two-hybrid assay, suggesting they could form heterodimers at some target promoters, as observed for some zinc cluster proteins involved in conferring drug resistance (Mamnun *et al.*, 2002; Akache *et al.*, 2004) or the Oaf1–Pip2 pair. Putative heterodimers could also be formed by an interaction with the other regulators Sip4, Cat8, and Ert1.

Role of the zinc cluster proteins Oaf1 and Pip2 in oleate utilization

The expression of the genes for fatty acid metabolism and peroxisome biogenesis is regulated by a combination of transcription factors (Smith *et al.*, 2007). For example, the zinc cluster protein Oaf3 is a weak repressor of oleate-responsive genes (Smith *et al.*, 2007). The zinc cluster proteins Oaf1 and Pip2 (Oaf2) have been extensively characterized and shown to mediate the response to oleate by binding as heterodimers to oleate response elements (consensus: $CGGN_3TNAN_{9–12}CCG$) found in the promoters of β-oxidation genes (Rottensteiner *et al.*, 1996, 1997). Although *OAF1* and *PIP2* coregulate the same genes, their expression is differentially regulated (Rottensteiner *et al.*, 1997). *OAF1* expression is constitutive whereas the expression of *PIP2* is positively autoregulated (Rottensteiner *et al.*, 1997). ChIP analysis has demonstrated that Oaf1 and Pip2 are found at common promoters (Karpichev *et al.*, 2008). The presence of Pip2 is also required for Oaf1 binding at most promoters tested. Moreover, successive ChIP assays (re-ChIP) with differently tagged Oaf1 and Pip2 have shown that these factors co-occupy the same target promoters. Thus, these data strongly suggest that an Oaf1–Pip2 heterodimer is mainly responsible for the activation of target genes. However, a few target genes (e.g. *FOX2*, *CTA1*) appear to be regulated by Oaf1, but not Pip2, suggesting activation by an Oaf1 homodimer (Trzcinska-Danielewicz *et al.*, 2008).

Binding of Oaf1–Pip2 to oleate response elements *in vivo* is increased by shifting cells from repressing (glucose) to derepressing conditions (glycerol), but is only marginally affected under inducing (oleate) conditions (Karpichev *et al.*, 2008). Thus, under derepressed conditions, Oaf1–Pip2 is constitutively bound to target promoters. Adr1 is also involved in regulating the expression of some genes for fatty acid oxidation and peroxisome biogenesis (Young *et al.*, 2003). ChIP experiments show that this factor is required for optimal binding of Oaf1–Pip2 at some promoters and vice versa (Karpichev *et al.*, 2008). Activation of the Oaf1/Pip2 heterodimer is mediated by direct binding of oleate to Oaf1 (Phelps *et al.*, 2006; Thakur *et al.*, 2009). Moreover, the presence of oleate results in hyperphosphorylation of Oaf1 and correlates with its transcriptional activity. The activation domain of Oaf1 was shown to interact with Med15 (Gal11), a subunit of the mediator complex that links transcriptional activators to general transcription factors and RNA polymerase II (Thakur *et al.*, 2009). From these various observations, a model for the mechanism of activation of Oaf1–Pip2 can be proposed. Under derepressing conditions, Oaf1 becomes phosphorylated by an unknown kinase favoring binding of the heterodimer to target genes, including the promoter of *PIP2*. Binding of oleate to Oaf1 would trigger a conformational change allowing interaction with Med15 (Gal11) and transcriptional activation. It is unclear as to why the presence of oleate results in hyperphosphorylation of Oaf1. One possibility is that this post-transcriptional modification may favor the interaction with Med15 (Gal11).

Mechanism of activation of transcription factors for utilization of nonfermentable carbons

As stated above, a key factor for the activation of glucose-repressed genes is the kinase Snf1 (also called Cat1) (for reviews, see Hardie *et al.*, 1998; Sanz, 2003, 2007; Hedbacker & Carlson, 2008). Briefly, Snf1 is activated under low glucose conditions and is a part of a complex that includes the activating subunit Snf4 (also called Cat3) and a third partner (Gal83, Sip1, or Sip2) (Erickson & Johnston, 1993; Yang *et al.*, 1994). The exact mechanism of Snf1 activation is still unclear, but it has been shown that the kinases Sak1 (Pak1), Tos3, and Elm1 are upstream effectors of Snf1 (Hong *et al.*, 2003; Sutherland *et al.*, 2003). These kinases phosphorylate Thr210 of the Snf1 activation loop. Pak1 activity is also required for nuclear localization of Snf1 (Hedbacker *et al.*, 2004). Tos3 activity is dispensable for responding to a sharp decrease of glucose levels, but is required for the activation of CSRE-containing genes with cells grown in ethanol/glycerol (Kim *et al.*, 2005). It is still not well understood as to how these kinases become activated under low glucose conditions. Moreover, other levels of regulation of Snf1 activity include autoinhibition (Jiang & Carlson, 1996; Leech *et al.*, 2003) as well as a potential control by dephosphorylation via the protein phosphatase complex I (Glc7/Reg1) (Sanz *et al.*, 2000).

Snf1 has multiple targets such as chromatin (histone H3), transcriptional activators, and repressors (Hedbacker & Carlson, 2008). For example, the transcriptional repressor Mig1 is a target of the Snf1 kinase. Phosphorylated Mig1 dissociates from the corepressor Ssn6-Tup1 protein complex and is exported to the cytoplasm through the exportin Msn5 (DeVit & Johnston, 1999; Smith *et al.*, 1999; Papamichos-Chronakis *et al.*, 2004), resulting in an increased expression of *CAT8*. Cat8 is also phosphorylated by the Snf1 kinase (Randez-Gil *et al.*, 1997). Convincing studies by Noël-Geoiris' group have shown that phosphorylation of a single serine residue in Cat8 from *S. cerevisiae* (or its homologue in *Kluyveromyces lactis*) is responsible for the activation of this factor (Charbon *et al.*, 2004). Snf1 phosphorylation of Sip4 correlates with its transcriptional activity (Lesage *et al.*, 1996). Similarly, hyperphosphorylation of Rds2 in ethanol is Snf1 dependent (Soontorngun *et al.*, 2007).

As stated above, transcriptional regulators of nonfermentable carbon utilization have distinct and overlapping targets. This observation raises the question of whether a given regulator requires a partner for binding at specific promoters or not. Is activation of target genes controlled at the step of binding of the regulator to specific DNA sequences in target promoters? A number of studies have addressed the interplay among these factors and diverse mechanisms appear to operate according to the factor and the target genes studied. For example, ChIP-chip results show that Rds2 is constitutively bound to the *PCK1* promoter, even under glucose conditions where (1) this gene is not expressed, (2) the Snf1 kinase is inactive, and (3) Cat8 is present at very low levels (Soontorngun *et al.*, 2007). Thus, phosphorylation of Rds2 by Snf1 is not required for binding of this factor at some promoters. However, under ethanol conditions, deletion of *CAT8* results in a modest decrease in binding of Rds2 at the *PCK1* promoter (twofold) while a more pronounced effect (over sixfold) is observed at the *FBP1* promoter, as determined by standard ChIP analysis

(Soontorngun *et al.*, 2007). These results provide an example of the interplay among these factors.

Additional recent studies have provided insights into the mechanism of activation of these nonfermentable gene regulators. For example, binding of Adr1 to the *ADH2* promoter as well as to other target genes (*CTA1*, *ACS1*, *GUT1*, and *POT1*) is Snf1 dependent (Young *et al.*, 2002). However, the cyclin-dependent kinase Pho85, but not Snf1, appears to be indirectly involved in Adr1 phosphorylation and inactivation (Kacherovsky *et al.*, 2008). Phosphorylation of Ser98, located in the DNA-binding domain of Adr1, is important for controlling the activity of this factor. For example, mimicking phosphorylation by mutating Ser98 to Asp decreases the binding affinity of Adr1, as assayed *in vitro* by an electrophoretic mobility shift assay and *in vivo* by ChIP (Kacherovsky *et al.*, 2008). As expected from the binding studies, the Asp98 mutant is transcriptionally inactive (Kacherovsky *et al.*, 2008).

A double deletion of the histone deacetylase genes *HDA1* and *RPD3* allows, even under repressive conditions, constitutive binding of Adr1 and Cat8 at target promoters such as *ADH2* (Tachibana *et al.*, 2007). Both Adr1 and Cat8 require the mediator complex as well as the chromatin remodeling complexes SWI/SNF and SAGA (Spt-Ada-Gcn5-acetyl transferase) for transcriptional activation (Biddick *et al.*, 2008). In a Δ*hda1*Δ*rpd3* strain, binding of these cofactors is observed while only marginal transcriptional activity is observed in the absence of Snf1 activation. In fact, binding of Adr1 and Cat8 is reduced in a triple deletion strain Δ*hda1*Δ*rpd3*Δ*snf1* (Tachibana *et al.*, 2007). Other results show that Snf1 mediates its effect after the binding of RNA polymerase II. Finally, a fusion of the DNA-binding domain of Adr1 to the Med15 (Gal11) component of the mediator bypasses the requirement for Snf1, SWI/SNF, and SAGA for the activation of *ADH2* (Young *et al.*, 2008). Taken together, Young's results suggest that the promoter of *ADH2* is accessible to Adr1, but that, under normal (repressing) conditions, Adr1 lacks the ability to interact with coactivators such as a mediator.

A model for the regulatory network of regulators of nonfermentable carbons

As stated above, the various transcriptional regulators of nonfermentable carbon metabolism have distinct and overlapping functions. Recent studies using genome-wide expression profiling and location analysis have provided additional useful information on the interplay among these transcription factors. Even though some of the experiments were not performed under the same conditions and may not be directly comparable, a model for the network of regulators of nonfermentable carbons is proposed in Fig. 3 that integrates various data. The expression of Rds2 does not vary significantly according to the carbon source and its activation correlates with its phosphorylation by the Snf1 kinase (Soontorngun *et al.*, 2007). Rds2 (and potentially Gsm1, as suggested by the ChIP analysis) increases the expression of *HAP4* encoding the limiting and activating subunit of the Hap2/3/4/5 complex (Soontorngun *et al.*, 2007; van Bakel *et al.*, 2008). The expression of *GSM1* is increased in nonfermentable carbons (Roberts & Hudson, 2006) by the Hap2/3/4/5 complex (Buschlen *et al.*, 2003), providing a putative autoregulatory loop between *HAP4* and *GSM1*.

Inactivation of Mig1 by Snf1 relieves the repression of *CAT8* expression, allowing the Hap2/3/4/5 complex to positively regulate the expression of *CAT8*. In agreement with this model, the expression of a CAT8-lacZ reporter was reduced five times when assayed in low glucose with a Δ*hap2* strain (Rahner *et al.*, 1996). Increased Cat8 levels and its activation by phosphorylation allow positive regulation of *SIP4*, which is probably also mediated by Rds2 because binding of this activator was detected at the *SIP4* promoter by ChIP (Soontorngun *et al.*, 2007). Remarkably, Cat8, Sip4, Rds2 Ert1, and Gsm1 all regulate the expression of *PCK1*. Regulation of Snf1 activity provides a means to control the whole network. In addition, Cat8 may provide a negative feedback loop in this system because expression of a CAT8-lacZ reporter is increased when assayed in a Δ*cat8* strain (Rahner *et al.*, 1996).

In recent years, significant progress has been made toward understanding the mechanism of transcriptional regulation of nonfermentable carbon utilization in *S. cerevisiae*. However, many questions remain to be answered. What is the exact mechanism of regulation of Snf1 activity? What is the exact role of Gsm1 and Ert1? Are there additional transcriptional regulators involved in this process?

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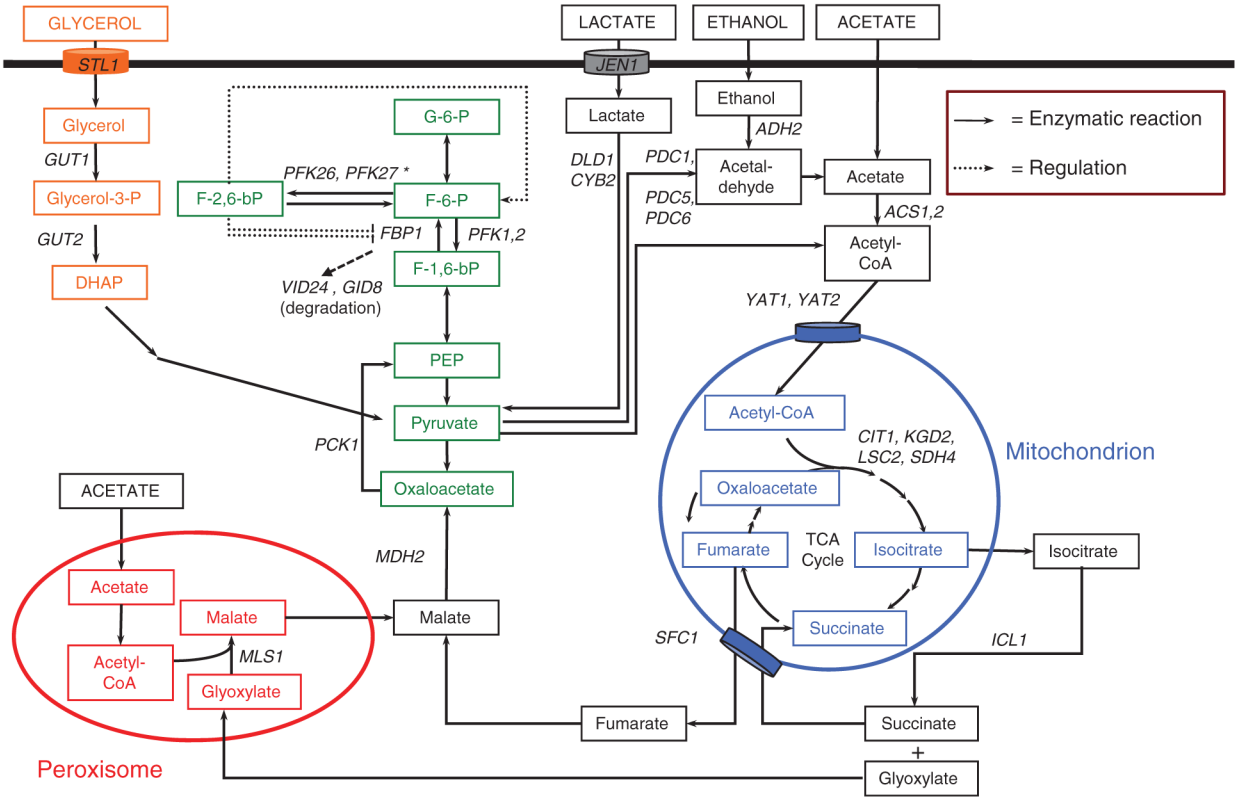

Fig. 1.

Metabolic pathways and genes involved in the utilization of nonfermentable carbons. Metabolic pathways for utilization of nonfermentable carbons are schematically shown as well as key genes involved in this process. The pathway for fatty acid metabolism was omitted (see Hiltunen *et al.*, 2003 for a review). Arrows with full lines correspond to enzymatic reactions while arrows with dashed lines correspond to regulatory steps. *STL1* and *JEN2* encode membrane transporters for glycerol and lactate, respectively. *SFC1* encodes a mitochondrial transporter for fumarate. More information for specific genes can be found at the yeast genome database (http://www.yeastgenome.org).

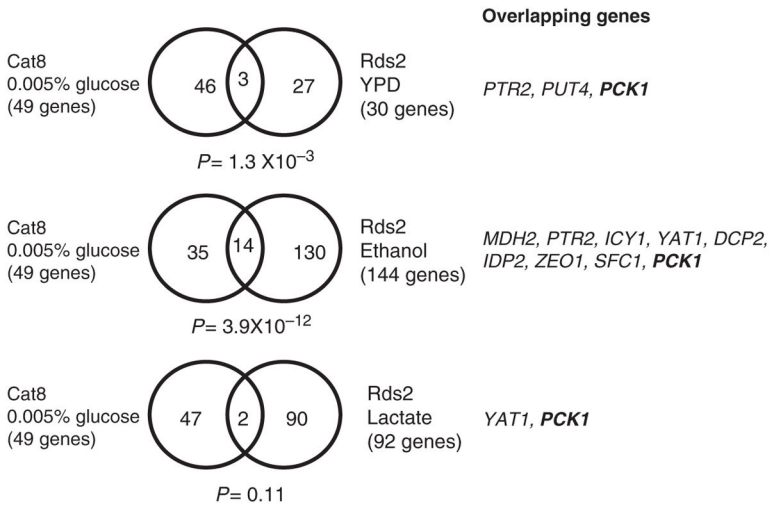

Fig. 2.

Limited overlap between Rds2 and Cat8 target genes. Cat8 target genes identified by ChIP-chip analysis under low glucose conditions (Tachibana *et al.*, 2005) were compared with those identified for Rds2 under ethanol (Soontorngun *et al.*, 2007) or lactate conditions (N. Soontorngun & B. Turcotte, unpublished data). *P*-values used for gene selection are indicated below Venn diagrams.

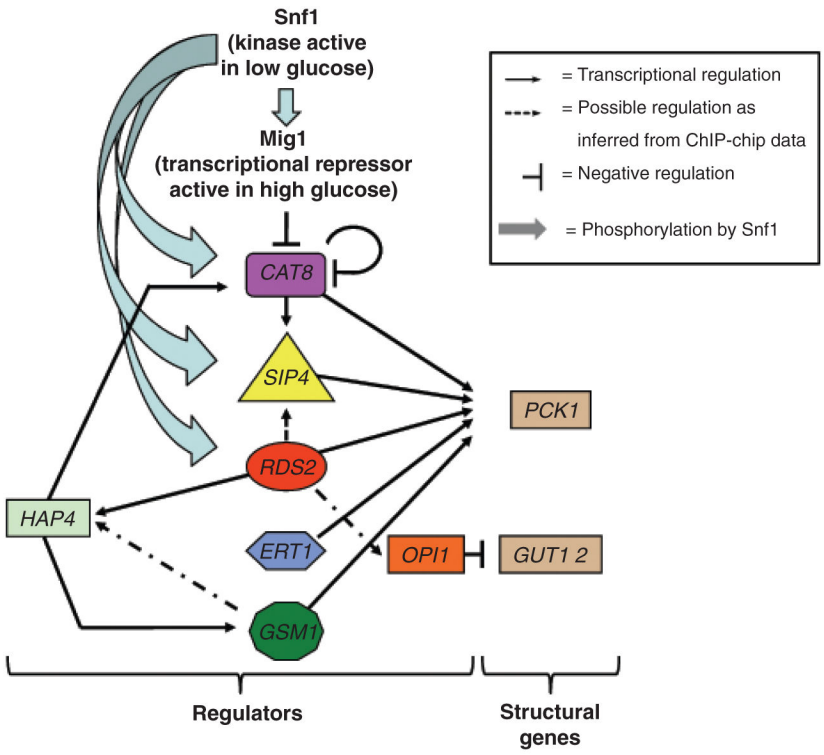

Fig. 3.

A model for the regulatory network of regulators of nonfermentable carbons. Low glucose levels activate the Snf1 kinase, resulting in phosphorylation and inactivation of the Mig1 repressor. Cat8, Sip4, and Rds2 are also substrates of Snf1. Rds2 and probably Gsm1 are activators of *HAP4*, whose gene product is a part of a complex involved in the positive control of *CAT8* and *GSM1*. Cat8 and most likely Rds2 are positive regulators of *SIP4*. *CAT8* expression is probably autoregulated. Cat8, Sip4, Rds2, Ert1, and Gsm1 are all transcriptional regulators of *PCK1* encoding a key gluconeogenic enzyme. ChIP analysis showed that Rds2 binds to the *OPI1* gene encoding a repressor of *GUT1* and *GUT2* expression involved in glycerol metabolism. See text for more details.

Table 1

Major transcriptional regulators of nonfermentable carbon utilization and their targets

Transcriptional regulator	Type of DNA-binding domain	Target genes
Adr1 (alcohol dehydrogenase regulator)	Cys_2His_2 zinc finger protein	Nonfermentable carbon metabolism (e.g. *ADH2*, *ACS1*, *GUT1*) Peroxisome biogenesis and fatty acids utilization (e.g. *POX1*, *PXA1*)
Cat8 (CATabolite repression)	Zinc cluster protein	Gluconeogenic genes (e.g. *PCK1*, *FBP1*) Glyoxylate cycle genes Transcription factor (*SIP4*)
Ert1 (ethanol regulator of translation)	Zinc cluster protein	*PCK1* Other targets unknown
Gsm1 (glucose starvation modulator)	Zinc cluster protein	Gluconeogenesis (*PCK1*, *FBP1*) Transcription factor (*HAP4*)
Hap1 (heme activator protein)	Zinc cluster protein	Respiration genes (e.g. *CYC1*, *CYC7*)
Hap2/3/4/5 (heme activator protein)	CCAAT-binding complex	Respiration genes (e.g. *CYC1*), TCA cycle
Oaf1 (oleate-activated transcription factor)	Zinc cluster protein	Fatty acids utilization (e.g. *POX1*, *FOX3*) Peroxisome biogenesis
Oaf3 (oleate-activated transcription factor)	Zinc cluster protein	Weak repressor of oleate-responsive genes
Pip2 (peroxisome induction pathway)	Zinc cluster protein	Fatty acids utilization (e.g. *POX1*, *FOX3*) Peroxisome biogenesis
Rds2 (regulator of drug sensitivity)	Zinc cluster protein	Gluconeogenic genes (e.g. *PCK1*, *FBP1*) Glyoxylate cycle genes (*MLS1*, TCA cycle genes) Transcription factors (*HAP4*, *SIP4*)
Sip4 (Snf1-interacting protein)	Zinc cluster protein	Gluconeogenic genes (e.g. *PCK1*)

For references, see text.

TCA, tricarboxylic acid.