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

Nitinpa Soontorngun a,*, Sirilak Baramee a, Chalinee Tangsombatvichit a, Piyasuda Thepnoka 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 Thanitthaya Road, Tha Kham, Bang Khunthian, Bangkok 10150, Thailand
b Biotechnology School of Bioresources and Technology, King Mongkut’s University of Technology Thonburi, 49 Thanitthaya Road, Tha Kham, Bang Khunthian, Bangkok 10150, Thailand
c Pilot Plant Development and Training Institute, King Mongkut’s University of Technology Thonburi, Bang Khunthian, 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

1. Introduction

Glucose is the preferred carbon source for the yeast Saccharomyces 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 C2H2 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 directly 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.

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* Corresponding author. Fax: +66 02 452 3479.
E-mail address: nitnipa.soo@kmutt.ac.th (N. Soontorngun).
source. In addition, Rds2 is a repressor of the negative gluconeogenic regulators PKK27 and VHD24 [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 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) [12]. The double deletion strain Δrds2Δacet8 (BY4741 background) was constructed by deleting the CAT8 open reading frame (ORF) in a Δrds2 strain using a PCR approach [13]. The phenotypic analysis of the Δrds2 strain was done in the FY73 background (MATa his3ΔΔ200 ura3Δ2) [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 as described by 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 OD600 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 bp 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.

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 SuperScriptIII 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−ΔΔCT 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 Δrds2 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 Δrds2 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 and linoleic acid (Fig. 1). In addition, a Δrds2 strain is unable to use lactate as a carbon source [15]. We also observed that the inability of a Δrds2 to grow on nonfermentable carbon sources varies according to

![Fig. 1. Impaired growth of the Δrds2 strain on nonfermentable carbon sources.](image-url)
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

<table>
<thead>
<tr>
<th>GO term</th>
<th>P-value</th>
<th>Fraction of the query</th>
<th>Fraction of the genome</th>
</tr>
</thead>
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<tr>
<td>Oxidation-reduction process</td>
<td>2.2 × 10⁻⁷</td>
<td>37/169</td>
<td>443/6359</td>
</tr>
<tr>
<td>Generation/precursors metabolites and energy</td>
<td>3.6 × 10⁻⁶</td>
<td>24/169</td>
<td>222/6359</td>
</tr>
<tr>
<td>Coenzyme metabolic process</td>
<td>2.9 × 10⁻⁵</td>
<td>19/169</td>
<td>157/6359</td>
</tr>
<tr>
<td>Energy derivation by oxidation of organic compounds</td>
<td>4.4 × 10⁻⁵</td>
<td>19/169</td>
<td>161/6359</td>
</tr>
<tr>
<td>Alcohol metabolic process</td>
<td>5.8 × 10⁻⁵</td>
<td>23/169</td>
<td>236/6359</td>
</tr>
<tr>
<td>Acetyl-CoA metabolic process</td>
<td>6.7 × 10⁻⁵</td>
<td>10/169</td>
<td>40/6359</td>
</tr>
<tr>
<td>Cellular respiration</td>
<td>2.2 × 10⁻⁴</td>
<td>15/169</td>
<td>111/6359</td>
</tr>
<tr>
<td>Aerobic respiration</td>
<td>5.2 × 10⁻⁴</td>
<td>13/169</td>
<td>88/6359</td>
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<tr>
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<td>8.8 × 10⁻⁴</td>
<td>8/169</td>
<td>30/6359</td>
</tr>
<tr>
<td>Tricarboxylic acid cycle</td>
<td>8.8 × 10⁻⁴</td>
<td>8/169</td>
<td>30/6359</td>
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<tr>
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<td>8/169</td>
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<tr>
<td>Acetyl-CoA metabolic process</td>
<td>6.7 × 10⁻⁵</td>
<td>10/169</td>
<td>40/6359</td>
</tr>
</tbody>
</table>
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 STBS 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 Δrds2 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 Δrds2 strain. Since Cat8 also binds to the genes listed above, qRT-PCR was also performed using a double deletion strain Δrds2 Δcat8. 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. Considering Table S3. Out of 152 promoters that are bound by Rds2, 20 (13%) 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 bound 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
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, VIG2, 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 auto-regulatory 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.

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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