Biochemical and Biophysical Research Communications 439 (2013) 501–505

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Nrg1 functions as a global transcriptional repressor of glucose-repressed genes through its direct binding to the specific promoter regions

Sung Bae Lee [a], Hyen Sam Kang [b], TaeSoo Kim [c,*]

[a] Department of Brain Science, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu 711-873, Republic of Korea
[b] School of Biological Sciences, Seoul National University, Seoul 151-747, Republic of Korea
[c] Department of Life Science and College of Natural Sciences, Ewha Womans University, Seoul 120-750, Republic of Korea

ARTICLE INFO

Article history:
Received 27 August 2013
Available online 8 September 2013

Keywords:
Nrg1
Glucose repression
SUC2
HXT2
Transcriptional regulation

ABSTRACT

Nrg1 is a zinc finger protein involved in the glucose repression of several glucose-repressed genes such as *STA1*, *SUC2*, and *GAL1*. Although the molecular details of the Nrg1-mediated repression of *STA1* have been partly characterized, it still remains largely unknown how Nrg1 regulates these multiple target genes. In this study, we show that Nrg1 mediates the glucose repression of *SUC2* and *HXT2* through its direct binding to the specific promoter regions; it binds to the −404 to −360 region of the *SUC2* promoter and the −957 to −810 region of the *HXT2* promoter. Nrg1 also interacts with the −380 to −250 region of the *PCK1* promoter, suggesting that it might also contribute to the *PCK1* repression. In addition, ChIP assays confirmed that Nrg1 associated with specific promoter regions of these glucose-repressed genes *in vivo*. Analysis of the DNA fragments to which it binds indicates that Nrg1 may recognize T/ACCCC sequence within the promoters of these glucose-repressed genes as well as in its own promoter. Collectively, our findings indicate that Nrg1 mediates the glucose repression of multiple genes through its direct binding to the specific promoter regions.

1. Introduction

The expressions of many genes are down-regulated when yeast cells are grown in a medium containing glucose [1–4]. This phenomenon, known as glucose repression, is mediated by several transcriptional repressors including Mig1, Mig2, and Nrg1 [3,5–10]. There are three groups of glucose-repressed genes: (1) genes encoding enzymes that metabolize other carbon sources (2) genes encoding enzymes required for gluconeogenesis, (3) genes involved in the Krebs cycle and in respiration. A well-known repressor, Mig1, binds to a GC-rich motif in the promoters of several glucose-repressed genes including *GAL1*, *SUC2*, and *MAL*, and recruits a co-repressor, the Ssn6-Tup1 complex to repress their transcription [6,10–14]. However, disruption of *MIG1* has little or no effect on glucose repression of other glucose-repressed genes, suggesting that the regulation of glucose repression may involve complicated contributions of multiple components.

Nrg1 was identified as a multicopy inhibitor of *STA1* that encodes extracellular glucoamylase I and is repressed in the presence of glucose [15,16]. It binds specifically to UAS1-1 (Upstream Activating Sequence 1-1) of the *STA1* promoter and protects two regions, the A and B boxes, from DNase I. It also recruits the Ssn6-Tup1 complex as in the case of Mig1 [16]. Previously, we reported that the two repressors, Nrg1 and Sfl1 bound to UAS1-1 and UAS2-2 of the *STA1* promoter, respectively and cooperated to repress *STA1* expression by recruiting the Srb8-11 complex [17]. Nrg1 is also known to inhibit haploid invasion and pseudohyphal differentiation by regulating expression of *FLO11* (encoding a cell surface glycoprotein) that has 5′ upstream regions similar to those of *STA1* and contains putative Nrg1 binding sites exactly matching to those of *STA1* [18–20]. Nrg1 has two tandem zinc finger motifs at its C-terminus, and Nrg2 contains motifs similar to those of Nrg1 [16,18,20]. These motifs in turn closely resemble those of Mig1 and Msn2 and are also related to those of the mammalian early growth response proteins, Egr-1/Zif268 and the Wilm's tumor protein [16,20,21]. In addition, it has been reported that Nrg1 also represses transcription of several glucose-repressed genes, *SUC2*, *GAL1*, and *GAL10* [10,20]. However it is still unclear whether Nrg1 binds directly to the promoters of these genes.

In this study we showed by Northern blot analysis and *lacZ*-reporter assays that Nrg1 repressed transcription of *SUC2* and *HXT2* in the presence of glucose. We also performed Electrophoretic Mobility Shift Assays (EMSAs) to test whether Nrg1 binds directly to the promoters of three glucose-repressed genes, *SUC2*, *HXT2*, and *PCK1* genes. We found that Nrg1 directly bound to the promoter regions of these three genes both *in vivo* and *in vitro*.

* Corresponding author. Fax: +82 2 3277 3760.
E-mail address: tskim@ewha.ac.kr (T. Kim).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.09.015

Finally, sequence analysis of the DNA fragments to which Nrg1 binds showed that the A box region is highly conserved, suggesting that the T/ACCCC sequence may be important for Nrg1 binding.

2. Materials and methods

2.1. Strains and media

Yeast cells were grown at 30 °C in YPD (1% yeast extract, 2% tryptone and 2% glucose) or synthetic medium containing 0.67% yeast nitrogen base without amino acids, supplemented with the appropriate amino acids and carbon sources (2% glucose or 2% glycerol/ethanol). To construct *nrg1Δ*::*KanMX6*, *KanMX6* was amplified by PCR with oligonucleotides containing 40 base pairs flanking the open reading frame of *NRG1*. The PCR products weEscherichia colire directly transformed into AN20-5b [16] and the mutation was confirmed by PCR using genomic DNA.

2.2. Plasmids

Plasmids used in this study are listed in Table 1. To generate the promoter-*lacZ* constructs, the promoter regions of *SUC2*, *HXT2*, and *PCK1* genes were amplified by PCR and the PCR products were subcloned into pLG-670z.

2.3. Northern blot analysis

Northern blot analyses were performed as described previously [16]. A 819-bp *Hind*III/*Xba*I fragment of the *SUC2* gene was used as a probe to detect *SUC2* mRNA. A 845-bp fragment of the *HXT2* gene and a 798-bp fragment of the *PCK1* gene were amplified by PCR and were similarly used as probes. A fragment of the *ACT1* gene was used as a control.

2.4. β-Galactosidase assay

β-galactosidase assay was conducted as described previously [15].

2.5. Electrophoretic mobility shift assays

EMSAs were performed as described previously. The DNA fragments used in EMSAs were amplified by PCR. GST-Nrg1 was expressed in *Escherichia coli* and purified essentially as described [16].

2.6. Chromatin immunoprecipitation assays

To detect protein–DNA interactions, chromatin immunoprecipitation assays were performed as described previously [22]. A transformant carrying pRS326-*NRG1*-HA was grown in 2% glucose to OD_{600} = 1.0 and exposed to formaldehyde (1%) to crosslink DNA and proteins. Sonicated extracts were prepared, and equal amounts of extract were incubated with mouse monoclonal α-HA antibody at 4 °C overnight. GammaBind G Sepharose beads (Amersham) were added to precipitate DNA–Nrg1–HA complexes. The beads were then washed four times and incubated at 65 °C overnight in elution buffer. DNA fragments were purified with a QiaQuick PCR column (Qiagen), and amplified by 30 cycles of PCR to detect the upstream regions of *STA1*, *SUC2*, *HXT2*, and *PCK1* that contain putative Nrg1 binding sites.

Table 1
Plasmids used in this study.

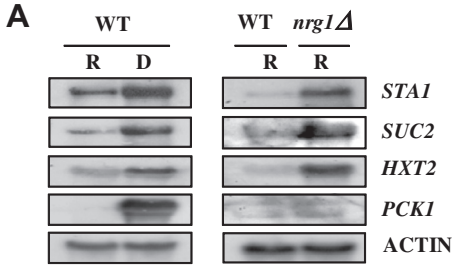
Plasmids	Phenotypes	References
pLG-669z	2 μ *URA3* UAS*CYC1-CYC1-lacZ*	[34]
pLG-670-z	2 μ *UAA3 CYC1-lacZ*	[34]
pLG-*STA1p*	2 μ *UAA3 STA1p-CYC1-lacZ*	[17]
pLG-*SUC2p*	2 μ *UAA3 SUC2p-CYC1-lacZ*	This study
pLG-*HXT2p*	2 μ *UAA3 HXT2p-CYC1-lacZ*	This study
pLG-*PCK1p*	2 μ *UAA3 PCK1p-CYC1-lacZ*	This study
pGEX-*NRG1*	*NRG1* in pGEX-4T-1	[16]
pRS326	2 μ *UAA3*	[35]
pRS326-*NRG1*-HA	2μ *UAA3 NRG1-HA*	This study

3. Results

3.1. nrg1Δ alleviates the glucose repression of SUC2 and HXT2 expression

To examine the effect of Nrg1 on the glucose repressed-genes *SUC2, HXT2* and *PCK1*, we compared their transcript levels in the wild type and a *nrg1Δ* mutant. In the wild type, transcript levels of *STA1, SUC2, HXT2* and *PCK1* were high when cells were grown in 2% glycerol/ethanol medium and significantly decreased in the presence of glucose (2%). However, in the *nrg1Δ* mutant, transcription of each of these genes, except *PCK1*, was noticeably elevated even in the presence of glucose (Fig. 1A).

To confirm this result, we examined the effect of Nrg1 on *lacZ* expression from the promoters of these genes. In the wild type, *lacZ* expression was completely inhibited in glucose-grown cells, but strongly increased in glycerol/ethanol-grown cells. In the *nrg1Δ* mutant, *lacZ* expression from the *STA1* and *HXT2* promoters was greatly increased in glucose medium, but *SUC2-lacZ* expression was only partially derepressed. Expression of *lacZ* from the *PCK1* promoter was not detected in either the wild type or the *nrg1Δ* mutant in the presence of glucose (Fig. 1B). These findings thus confirm that Nrg1 acts as a transcriptional repressor in the glucose repression of *STA1, SUC2* and *HXT2*.



B

	WT	*nrg1Δ*	WT
	R	R	D
STA1p-lacZ	15.49	62.41	153.93
SUC2p-lacZ	0.21	10.96	106.59
HXT2p-lacZ	6.50	47.73	75.26
PCK1p-lacZ	0.36	0.32	68.71

Fig. 1. The effect of *NRG1* mutation on the transcription of glucose-repressed genes. (A) Wild type and *nrg1Δ* cells were grown in synthetic medium containing 2% glucose [R] or 2% glycerol/ethanol [D]. Total RNA was prepared for Northern blot analysis, and the yeast actin gene (*ACT1*) was used as internal control. (B) Wild type and *nrg1 Δ* cells were transformed with plasmids carrying promoter-*lacZ* fusions and the transformants were grown to mid-log phase in synthetic medium containing 2% glucose [R] or 2% glycerol/ethanol [D]. In each case the β-galactosidase activity of three independent transformants was determined.

3.2. Nrg1 binds to the specific regions of the SUC2 promoter in vitro

Nrg1 binds to UAS1-1 region of the *STA1* promoter under repressed conditions [16], and our above findings show that Nrg1 is also involved in the glucose repression of *SUC2* and *HXT2*. We therefore performed electrophoretic mobility shift assays (EMSAs) to determine whether Nrg1 directly binds to the *SUC2* promoter. The promoter region of *SUC2* (−841 to +202) was subdivided into three fragments (Fig. 2A: a, b and c). Each fragment was used together with purified recombinant GST-Nrg1. As shown in Fig. 2B, Nrg1 bound to the SUC2-b fragment, and we confirmed the specificity of this binding in a competition assay using unlabeled SUC2-b fragment (Fig. 2C). To localize more accurately the binding site of Nrg1, we performed EMSAs using two halves of the SUC2-b fragment, SUC2-b1 and SUC2-b2. A shifted band was only detected with SUC2-b2 (Fig. 2D). Finally, EMSAs using three sub-fragments of SUC2-b2 showed that Nrg1 binds to the −404 to −360 bp region (SUC2-b2-1) of the *SUC2* promoter (Fig. 2E). Notably, this region does not overlap the binding site (−499 to −431) of Mig1 [8,11,13].

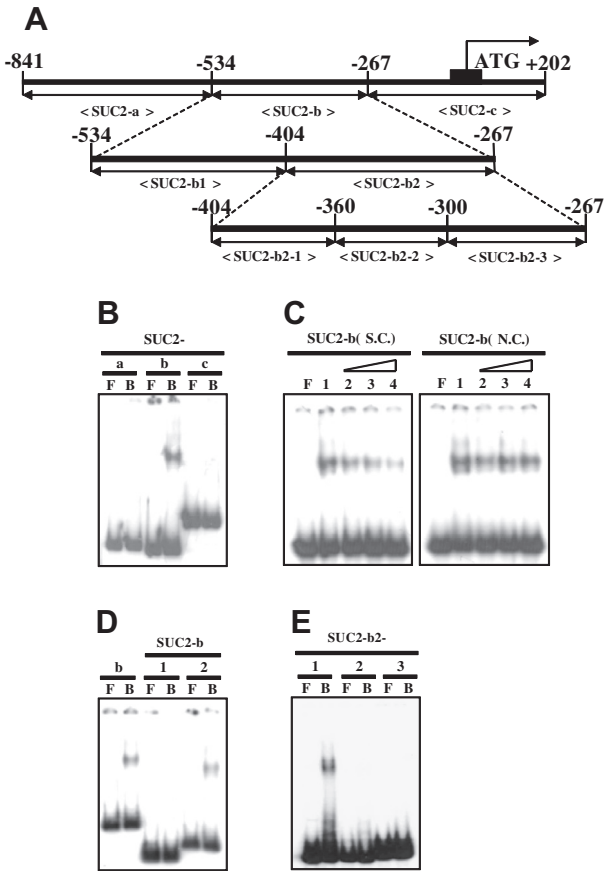


Fig. 2. Nrg1 binds directly to the *SUC2* promoter. (A) Structure of the DNA fragments of the *SUC2* promoter used in the EMSAs. (B) The three fragments, SUC2-a, SUC2-b, and SUC2-c were used as probes. Lanes; F, no added protein; B, reactions with 1ug of GST-Nrg1. (C) Competitive EMSA assays performed with 1μg of GST-Nrg1. Lanes in left panel: F, no added protein; 1, no specific competitor; 2–4, increasing amounts of specific competitor (16-, 32-, and 64-fold molar excess of unlabeled SUC2-b). Lanes in light panel: F, no added protein; 1, no nonspecific competitor; 2–4, increasing amounts of nonspecific competitor (16-, 32-, and 64-fold molar excess of unlabeled SUC2-a). (D) The two fragments, SUC2-b1 and SUC2-b2, were used as probes. Lanes: F, no added protein; B, reactions with 1ug of GST-Nrg1. (E) The three fragments, SUC2-b2-1, SUC2-b2-2, and SUC2-b2-3, were used as probes. Lanes: F, no added protein; B, reactions with 1ug of GST-Nrg1.

3.3. Binding of Nrg1 to the promoters of HXT2 and PCK1 in vitro

To investigate whether Nrg1 also binds to the promoter of *HXT2*, we carried out EMSAs with three fragments of the promoter (−957 to +23) (Fig. 3A). Nrg1 bound specifically to the HXT2-a fragment (Fig. 3B and 3C). Although Nrg1 bound slightly to HXT2-b, the shifted complex disappeared when either specific or nonspecific competitor was added (Fig. 3C). To define further the region to which Nrg1 binds, three sub-fragments of HXT2-a were used as probes. Shifted bands were detected with both HXT2-a1 and HXT2-a2, but binding was stronger to HXT2-a1 (Fig. 3D).

Next, although the *nrg1Δ* mutation did not affect *PCK1* transcription, we tested for the binding of Nrg1 to the *PCK1* promoter

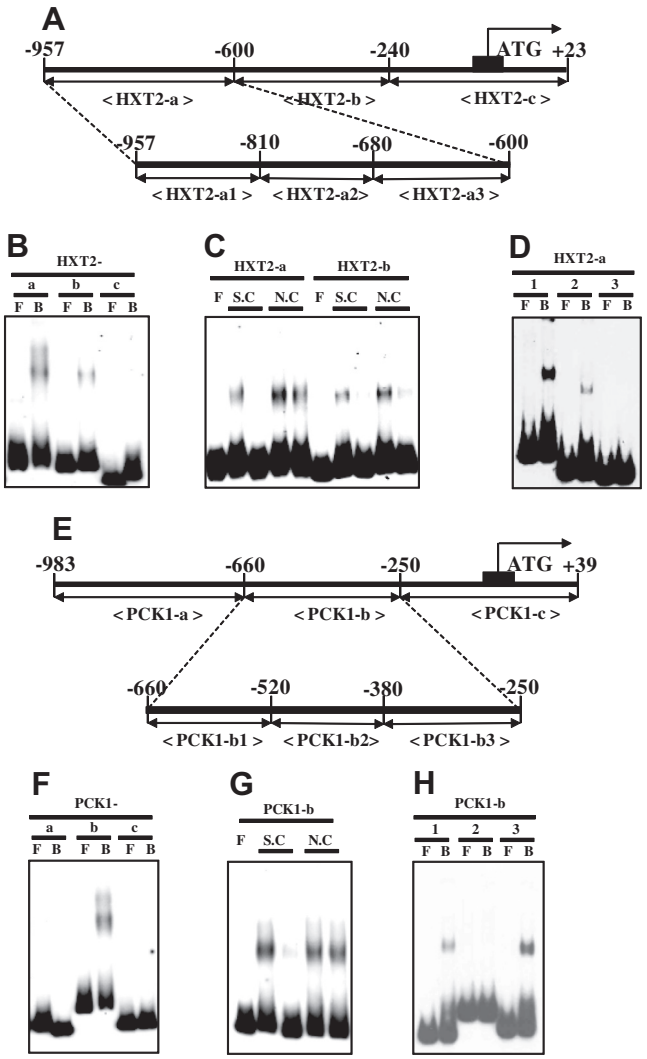


Fig. 3. Specific binding of Nrg1 to the *HXT2* and *PCK1* promoters. (A) Structure of the DNA fragments of the *HXT2* promoter used in EMSAs. (B) The three fragments, HXT2-a, HXT2-b, and HXT2-c used as probes. Lanes; F, no added protein; B, reactions with 1ug of GST-Nrg1. (C) Competitive EMSA assays with 1μg of GST-Nrg1. Lanes: F, no added protein; S.C, specific competitor (64X unlabeled HXT2-a or 64× unlabeled HXT2-b); N.C, specific competitor (64× unlabeled HXT2-c). (D) The three fragments, HXT2-a1, HXT2-a2, and HXT2-a3, used as probes. Lanes: F, no added protein; B, reactions with 1ug of GST-Nrg1. (E) Structure of the DNA fragments of the *PCK1* promoter used in EMSAs. (F) The three fragments, PCK1-a, PCK1-b, and PCK1-c used as probes. Lanes; F, no added protein; B, reactions with 1μg of GST-Nrg1 (G) Competitive EMSA assays were performed with 1ug of GST-Nrg1. Lanes: F, no added protein; S.C, addition of specific competitor (64× unlabeled PCK1-b); N.C, addition of specific competitor (64× unlabeled PCK1-c). (H) The three fragments, PCK1-b1, PCK1-b2, and PCK1-b3, were used as probes. Lanes: F, no added protein; B, reactions with 1ug of GST-Nrg1.

because we could not exclude the possibility that *PCK1* expression can be repressed by some other repressor(s) in combination with Nrg1. Actually, this mode of transcriptional control has been verified for other transcription factors; Mig2 repressor binds to the *SUC2* promoter but deletion of *MIG2* alone has no effect on *SUC2* expression [7,10]. However, a *mig1Δmig2Δ* double mutant had a higher level of *SUC2* expression than a *mig1Δ* single mutant [7,10]. Another example is Sfl1 transcription factor: it was also reported that Sfl1 repressor was implicated in the glucose repression of *SUC2* expression. Deletion of *SFL1* showed only slightly increased *SUC2* expression but a *mig1Δsfl1Δ* double mutant had strongly increased invertase activity [23].

Of the three promoter fragments tested (Fig. 3E), Nrg1 only bound to PCK1-b (Fig. 3F), and competition assays confirmed that the binding was specific (Fig. 3G). We used three sub-fragments to explore this binding further and found that Nrg1 bound to both PCK1-b1 and PCK1-b3 (Fig. 3H). However, binding to PCK1-b1 was significantly reduced by addition of nonspecific competitor, indicating that binding to PCK1-b1 was non-specific. Thus, although *PCK1* expression was not affected by *NRG1* mutation, the specific binding of Nrg1 to the *PCK1* promoter raises the possibility that Nrg1 might somehow contribute to the glucose repression of *PCK1*.

3.4. Nrg1 interacts with the promoter regions of STA1, SUC2, HXT2 and PCK1 in vivo

Since Nrg1 binds to specific regions within the *STA1*, *SUC2*, *HXT2*, and *PCK1* promoters *in vitro*, we next performed chromatin immunoprecipitation (ChIP) assays to test whether it indeed interacts with these promoters *in vivo*. HA-tagged Nrg1 was expressed from the native *NRG1* promoter, and cells carrying a plasmid expressing Nrg1-HA were grown to mid-log phase in 2% glucose medium. As shown in Fig. 4A, Nrg1 interacts with the promoters of *STA1*, *SUC2*, *HXT2* and *PCK1* whereas, as expected, it did not interact with the *KSS1* open reading frame as a negative control. This result indicates that Nrg1 binds to the specific promoter regions of glucose-repressed genes *in vivo*.

3.5. Sequence analysis of the putative Nrg1 binding sites

It has been previously shown that Nrg1 binds to UAS1-1 of the *STA1* promoter and protects two regions, designated the A and B boxes, from DNase I [16]. It was also suggested that the sequence CCCCT in A box and CCCTC in B box might be important for its binding [16]. To examine whether there are conserved A and B boxes in the *SUC2*, *HXT2*, and *PCK1* promoters, we analyzed the nucleotide sequences of the fragments of these promoters bound by Nrg1. As shown in Fig. 4B, there are highly conserved A boxes in the promoters of *STA1*, *SUC2*, *HXT2* and *PCK1*, whereas there are no similarly conserved B boxes. These findings suggest that the A box region may be more important for Nrg1 binding to these promoters. According to the previous report, Nrg1 also bound to its own promoter and supposedly, this in turn regulates its own transcription [24]. We therefore also analyzed the upstream region of *NRG1* and found a putative Nrg1 binding site which is very similar to that of the *STA1* promoter (Fig. 4B). Collectively, these results suggest that Nrg1 represses transcription of multiple glucose-repressed genes through its direct binding to the specific promoter regions.

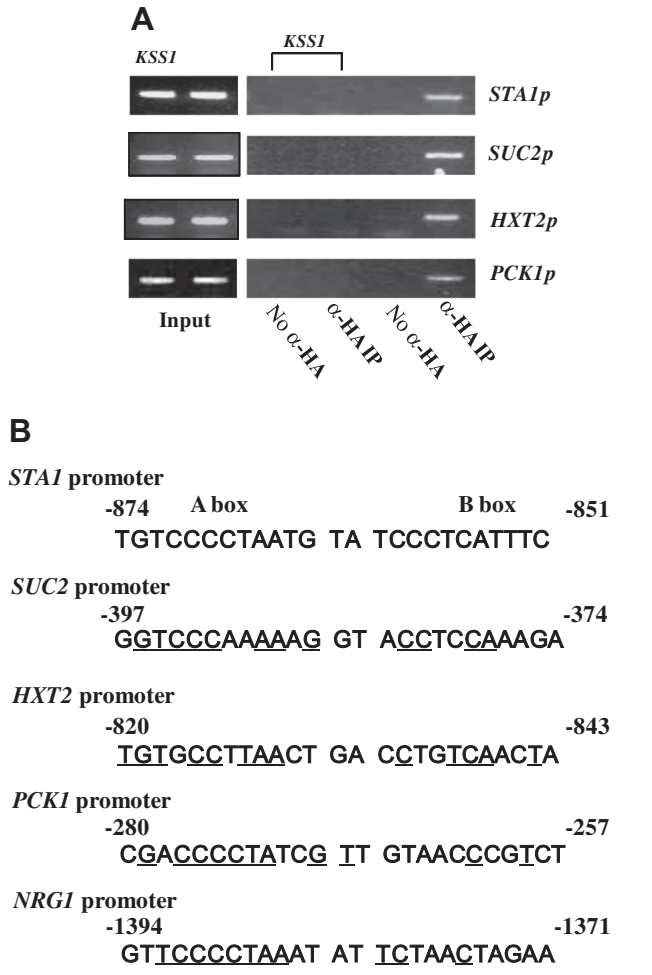


Fig. 4. Nrg1 binds to the promoter regions of glucose-repressed genes *in vivo*. (A) Wild type cells were transformed with a plasmid expressing HA tagged Nrg1 from the native *NRG1* promoter. Transformants were grown to mid-log phase in medium containing 2% glucose. Cells were treated with formaldehyde to cross-link Nrg1-HA and DNA and total extracts prepared. DNA fragments immunoprecipitated with or without addition of α-HA antibody were analyzed by PCR. As a control, interaction between *KSS1* DNA and Nrg1-HA was tested. (B) Sequence analysis of the putative Nrg1 binding sites in the *SUC2*, *HXT2*, *PCK1*, and *NRG1* promoters. The A box of the *STA1* promoter is highly conserved in all four promoters. Sequences identical to those of the *STA1* promoter (UAS1-1) are underlined.

4. Discussion

We have previously identified Nrg1 in yeast as a multicopy inhibitor of *STA1* encoding an enzyme required for starch utilization and shown its binding to UAS1-1 of the *STA1* promoter as a mechanism [16]. In the current study, we show that *nrg1Δ* mutation relieves the glucose repression of *STA1*, *SUC2* and *HXT2*, and that Nrg1 directly binds to the specific promoter regions of these genes. Furthermore, Nrg1 also binds to the *PCK1* promoter even though the *nrg1Δ* mutation has no effect on *PCK1* expression suggesting that Nrg1 may play a minor or supplementary role in the glucose repression of *PCK1*. These results collectively implicate that Nrg1 may function as a general transcriptional repressor of glucose-repressed genes.

Both Nrg1 and Mig1 interact with the Ssn6-Tup1 complex to give the glucose repression [14,16]. In addition, we have reported that Nrg1 also interacts with the Srb8-11 complex which is critical for the glucose repression of *STA1* expression [17]. These findings indicate that Nrg1 can recruit either the Ssn6-Tup1 complex or Srb8-11 complex to repress transcription of glucose-repressed genes. Recently, genome-wide association and gene expression analyses revealed that Ssn6 or Tup1 bound to gene promoters that we tested here and that loss of this complex resulted in derepression of these genes in repressed conditions [25,26]. These data suggest that recruitment of the Ssn6–Tup1 complex to these genes might require Nrg1.

Previously, Kuchin et al. have reported that Nrg1 also inhibits haploid invasion and pseudohyphal differentiation by repressing transcription of *FLO11*, which has a putative Nrg1 binding site identical to that of *STA1* [18,27,28]. They proposed as a possible mechanism that the function of Nrg1 was inhibited by Snf1 kinase, which is known to interact with Nrg1 [18,20]. However, it is still unclear whether Snf1 regulates Nrg1 function directly. Notably, the cellular location of Nrg1 was not affected by differences in carbon sources [20]. In case of Mig1, its function is regulated by Snf1 kinase, and indeed Mig1 is localized to the nucleus in the presence of glucose and binds to the promoter regions of its target genes; when glucose is depleted, Snf1 is activated and thereafter phosphorylates Mig1 to prevent its translocation to the nucleus [7,29–33]. On the other hand, we proposed previously that the function of Nrg1 might be regulated at the transcriptional level because transcription of *NRG1* increased about 6-fold in the presence of glucose [16]. Consistently, the level of Nrg1 protein was about 3.5-fold higher in glucose-grown cells than in glycerol/ethanol-grown cells [17]. Interestingly, *NRG1* transcript levels were increased in the absence of *SSN6* or *TUP1*, indicating that Nrg1 may utilize the Ssn6–Tup1 complex to repress its own transcription [26]. These results suggest that Nrg1 function might be regulated primarily at the transcriptional level, which interestingly involves its own molecular activity.

Park et al. suggested that two regions of UAS1-1 of the *STA1* promoter, the A and B boxes, were important for Nrg1 binding [16]. The putative Nrg1 binding sequences, CCCCT and CCCTC, differ from the Mig1 binding sequence, (G/C)(C/T)GG(G/A)G [16]. Interestingly, the Nrg1 binding site does not overlap that of Mig1 in the *SUC2* promoter, suggesting that Nrg1 binding to the promoter regions of glucose-repressed genes is likely independent of binding of other repressor(s).

We show here that the A box region is highly conserved in the sequences of the promoters of multiple glucose-repressed genes to which Nrg1 binds. Moreover the *NRG1* promoter also contains a putative Nrg1 binding sequence, thus supporting the previous evidence that Nrg1 binds to its own promoter and regulates its own transcription. All of these results indicate that Nrg1 recognizes and binds to the conserved sequences in the promoter regions of multiple glucose-repressed genes to repress transcription.

Acknowledgments

This work was partially supported by the DGIST MIREBrain and R&D Program of the ministry of science, ICT & future planning, Science and Technology of Korea (13-BD-0402, 13-HRLA-1) (S.B.L.) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A1008634) (T.K.).

References

[1] J.M. Gancedo, Carbon catabolite repression in yeast, Eur. J. Biochem. 206 (2) (1992) 297–313.
[2] J.M. Gancedo, Yeast carbon catabolite repression, Microbiol. Mol. Biol. Rev. 62 (2) (1998) 334–361.
[3] H. Ronne, Glucose repression in fungi, Trends Genet. 11 (1) (1995) 12–17.
[4] R.J. Trumbly, Glucose repression in the yeast *Saccharomyces cerevisiae*, Mol. Microbiol. 6 (1) (1992) 15–21.
[5] A. Alberti et al., MIG1-dependent and MIG1-independent regulation of GAL gene expression in *Saccharomyces cerevisiae*: role of Imp2p, Yeast 20 (13) (2003) 1085–1096.
[6] Z. Hu et al., MIG1-dependent and MIG1-independent glucose regulation of MAL gene expression in *Saccharomyces cerevisiae*, Curr. Genet. 28 (3) (1995) 258–266.
[7] L.L. Lutfiyya, M. Johnston, Two zinc-finger-containing repressors are responsible for glucose repression of SUC2 expression, Mol. Cell. Biol. 16 (9) (1996) 4790–4797.
[8] J.O. Nehlin, M. Carlberg, H. Ronne, Control of yeast GAL genes by MIG1 repressor: a transcriptional cascade in the glucose response, EMBO J. 10 (11) (1991) 3373–3377.
[9] L.G. Vallier, M. Carlson, Synergistic release from glucose repression by mig1 and ssn mutations in *Saccharomyces cerevisiae*, Genetics 137 (1) (1994) 49–54.
[10] H. Zhou, F. Winston, NRG1 is required for glucose repression of the SUC2 and GAL genes of *Saccharomyces cerevisiae*, BMC Genet. 2 (2001) 5.
[11] D.W. Griggs, M. Johnston, Regulated expression of the GAL4 activator gene in yeast provides a sensitive genetic switch for glucose repression, Proc. Natl. Acad. Sci. USA 88 (19) (1991) 8597–8601.
[12] C.A. Keleher et al., Ssn6-Tup1 is a general repressor of transcription in yeast, Cell 68 (4) (1992) 709–719.
[13] M. Lundin, J.O. Nehlin, H. Ronne, Importance of a flanking AT-rich region in target site recognition by the GC box-binding zinc finger protein MIG1, Mol. Cell. Biol. 14 (3) (1994) 1979–1985.
[14] M.A. Treitel, M. Carlson, Repression by SSN6-TUP1 is directed by MIG1, a repressor/activator protein, Proc. Natl. Acad. Sci. USA 92 (8) (1995) 3132–3136.
[15] J.H. Ahn, S.H. Park, H.S. Kang, Inactivation of the UAS1 of STA1 by glucose and STA10 and identification of two loci, SNS1 and MSS1, involved in STA10-dependent repression in *Saccharomyces cerevisiae*, Mol. Gen. Genet. 246 (5) (1995) 529–537.
[16] S.H. Park et al., Nrg1 is a transcriptional repressor for glucose repression of STA1 gene expression in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 19 (3) (1999) 2044–2050.
[17] T.S. Kim, S.B. Lee, H.S. Kang, Glucose repression of STA1 expression is mediated by the Nrg1 and Sfl1 repressors and the Srb8-11 complex, Mol. Cell. Biol. 24 (17) (2004) 7695–7706.
[18] S. Kuchin, V.K. Vyas, M. Carlson, Snf1 protein kinase and the repressors Nrg1 and Nrg2 regulate FLO11, haploid invasive growth, and diploid pseudohyphal differentiation, Mol. Cell. Biol. 22 (12) (2002) 3994–4000.
[19] V.K. Vyas et al., Snf1 kinases with different beta-subunit isoforms play distinct roles in regulating haploid invasive growth, Mol. Cell. Biol. 23 (4) (2003) 1341–1348.
[20] V.K. Vyas, S. Kuchin, M. Carlson, Interaction of the repressors Nrg1 and Nrg2 with the Snf1 protein kinase in *Saccharomyces cerevisiae*, Genetics 158 (2) (2001) 563–572.
[21] J.O. Nehlin, H. Ronne, Yeast MIG1 repressor is related to the mammalian early growth response and Wilms' tumour finger proteins, EMBO J. 9 (9) (1990) 2891–2898.
[22] A. Hecht, S. Strahl-Bolsinger, M. Grunstein, Spreading of transcriptional repressor SIR3 from telomeric heterochromatin, Nature 383 (6595) (1996) 92–96.
[23] W. Song, M. Carlson, Srb/mediator proteins interact functionally and physically with transcriptional repressor Sfl1, EMBO J. 17 (19) (1998) 5757–5765.
[24] T.I. Lee et al., Transcriptional regulatory networks in *Saccharomyces cerevisiae*, Science 298 (5594) (2002) 799–804.
[25] B.J. Venters et al., A comprehensive genomic binding map of gene and chromatin regulatory proteins in *Saccharomyces*, Mol. Cell 41 (4) (2011) 480–492.
[26] T.L. Lenstra et al., The specificity and topology of chromatin interaction pathways in yeast, Mol. Cell 42 (4) (2011) 536–549.
[27] B.R. Braun, D. Kadosh, A.D. Johnson, NRG1, a repressor of filamentous growth in *Candida albicans*, is down-regulated during filament induction, EMBO J. 20 (17) (2001) 4753–4761.
[28] A.M. Murad et al., NRG1 represses yeast-hypha morphogenesis and hypha-specific gene expression in *Candida albicans*, EMBO J. 20 (17) (2001) 4742–4752.
[29] J.L. Celenza, M. Carlson, Cloning and genetic mapping of SNF1, a gene required for expression of glucose-repressible genes in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 4 (1) (1984) 49–53.
[30] J.L. Celenza, M. Carlson, A yeast gene that is essential for release from glucose repression encodes a protein kinase, Science 233 (4769) (1986) 1175–1180.
[31] M.J. De Vit, J.A. Waddle, M. Johnston, Regulated nuclear translocation of the Mig1 glucose repressor, Mol. Biol. Cell 8 (8) (1997) 1603–1618.
[32] M.A. Treitel, S. Kuchin, M. Carlson, Snf1 protein kinase regulates phosphorylation of the Mig1 repressor in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 18 (11) (1998) 6273–6280.
[33] L.L. Lutfiyya et al., Characterization of three related glucose repressors and genes they regulate in *Saccharomyces cerevisiae*, Genetics 150 (4) (1998) 1377–1391.
[34] L. Guarente, M. Ptashne, Fusion of *Escherichia coli* lacZ to the cytochrome c gene of *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA 78 (4) (1981) 2199–2203.
[35] T.W. Christianson et al., Multifunctional yeast high-copy-number shuttle vectors, Gene 110 (1) (1992) 119–122.