The MIG1 repressor from *Kluyveromyces lactis*: cloning, sequencing and functional analysis in *Saccharomyces cerevisiae*

Jean-Pol Cassart[a,*], Isabelle Georis[a], Jonas Östling[b,**], Hans Ronne[b,**], Jean Vandenhaute[a]

[a]*Unité de Recherches Concertées en Biologie Moléculaire, Laboratoire de Génétique Moléculaire, Facultés Notre-Dame de la Paix, rue de Bruxelles 61, B-5000 Namur, Belgium*

[b]*Ludwig Institute for Cancer Research, Uppsala Branch, Box 595, S-75124 Uppsala, Sweden*

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Abstract **Sequence comparisons between *Saccharomyces cerevisiae* ScMig1 and *Aspergillus nidulans* CREA proteins allowed us to design two sets of degenerate primers from the conserved zinc finger loops. PCR amplification on *Kluyveromyces marxianus* and *K. lactis* genomic DNA yielded single products with sequences closely related to each other and to the corresponding regions of ScMig1 and CREA. The *KlMIG1* gene of *K. lactis* was cloned from a genomic library using the *K. marxianus* PCR fragment as probe. *KlMIG1* encodes a 474-amino acid protein 55% similar to ScMig1. Besides their highly conserved zinc fingers, the two proteins display short conserved motifs of possible significance in glucose repression. Heterologous complementation of a *mig1* mutant of *S. cerevisiae* by the *K. lactis* gene demonstrates that the function of the Mig1 protein is conserved in these two distantly related yeasts.**

Key words: MIG1; CREA; Zinc finger protein; Glucose repression; *Kluyveromyces lactis*

1. Introduction

In *Saccharomyces cerevisiae*, the MIG1 protein (ScMig1) has been identified as a downstream specific effector of glucose repression [1,2]. It is a C_2H_2 zinc finger protein which binds to well-characterized GC-rich sites and has strong similarity to the CREA regulator of *Aspergillus nidulans* and to the mammalian SP1, Krox/Egr and Wilms' tumour proteins [3,4]. According to the current model, in the presence of glucose, ScMig1 represses transcription by recruiting the Ssn6-Tup1 complex to the target promoter [5–7]. In the absence of glucose, the repressor function of Mig1 is thought to be inhibited, directly or indirectly by the Snf1 kinase. Several glucose-repressed genes are controlled by Mig1, directly and/or indirectly by transcriptional repression of a transcription activator. For example, in the case of the *GAL1* gene, Mig1 acts at two levels, directly on the *GAL1* promoter but also indirectly by repression of *GAL4* expression [8].

In the distantly related yeast *Kluyveromyces lactis*, some distinct features are observed in glucose repression, in particular with respect to the *GAL* genes. For instance, expression of *KlLAC9* (the *K. lactis* counterpart of *ScGAL4*) is sharply autoregulated and its promoter displays no putative MIG1-binding site [9,10]. Moreover, in addition to its galactokinase activity, the *K. lactis GAL1* gene product has a regulatory function required for the induction pathway, upstream of KlLAC9 [11]. Interestingly, a potential MIG1-binding site is found in the *GAL1* promoter of *K. lactis* suggesting the involvement of a Mig1-like protein in glucose repression of galactose-lactose catabolism in *K. lactis*.

The *SUC2* gene encoding invertase in *S. cerevisiae*, on the other hand, displays two MIG1-binding sites in its promoter [3] and two putative MIG1 sites are also found [12] upstream of the *INU1* gene encoding inulinase (a closely related enzyme) in *Kluyveromyces marxianus* [13], suggesting that the latter gene also may be repressed by a Mig1 homologue.

In this paper, we report the cloning and sequencing of the *K. lactis MIG1* gene and we show that the encoded protein is functional in *S. cerevisiae*.

2. Materials and methods

2.1. Strains and plasmids

For the 2-deoxyglucose and β-galactosidase assays, the following strain and plasmids were used. The *S. cerevisiae* strain was H497 (*MATa SUC2 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1::hisG ura3-1 mig1-δ3*) which is congenic to W303-1A [14]. The plasmid carrying the *SUC2-lacZ* reporter fusions was pJO25 [14]. The plasmid containing *KlMIG1* was pGIKL2. *KlMIG1* was subcloned from pGIKL2-3 on a 3800-bp *Eco*RI-*Eco*RV fragment into pRS313 plasmid [15]. The plasmid containing *ScMIG1* was pJO101. *ScMIG1* was subcloned from pMIG1 [3] on a 2160-bp *Sac*I fragment in the polylinker *Sac*I site of pHR68 [16]. In the resulting plasmid, pMC120, a 1764-bp *Bam*HI *HIS3* marker was inserted between the unique *Xba*I and *Nsi*I sites replacing *URA3* marker. The vector control was pJO99. The *URA3* marker of pHR68 [16] was replaced by a *HIS3* marker as described for pJO101.

2.2. General DNA techniques

Routine DNA manipulations were performed essentially as described by Sambrook et al. [17], using the *Escherichia coli* xl1 blue strain (Stratagene). The *K. lactis* genomic library was screened by filter hybridization using nylon filters washed at 68°C using 0.2 × SSC/0.1% SDS as final washing buffer. Sequencing was performed on denatured plasmid DNA with the T7 sequencing kit (Pharmacia) using synthetic oligonucleotides.

2.3. Design of degenerate primers

The amino acid comparisons and the sequences of the 64-fold degenerate primers are shown in Fig. 1. The primers covered 6 codons and were flanked by an *Eco*RI site at their 5′ end for cloning purposes. As the codon usage is very similar in *K. lactis* and *S. cerevisiae* [18], the codons were selected according to the codon usage described by Bennetzen and Hall [19]. At the 3′ end of the left primers, we included the first two bases of a threonine codon. The identical first two nucleotides of serine or arginine were included at the 5′ end of the right primers.

2.4. PCR amplification

The 100-μl PCR reaction contained 250 pmol of each of the degener-

*Corresponding author. Fax: (32) (81) 72 44 20.
E-mail: jpcassart@cc.fundp.ac.be

**Present address:* Department of Medical Immunology and Microbiology, Uppsala University, Uppsala Biomedical Center, Box 582, S-75123 Uppsala, Sweden.

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ate primers, incubation buffer (20 mM Tris-HCl, pH 8.4, 2 mM $MgCl_2$, 50 mM KCl), dNTP to a final concentration of 0.2 mM, 2.5 U Taq DNA polymerase (Gibco-BRL) and 0.1 μg of genomic DNA. Prior to amplification, the template was initially denatured 3 min at 94°C. This was followed by 35 cycles at 94°C for 1 min 15 s, 55°C for 1 min 15 s and 72°C for 3 min. The reaction was terminated after a final 10-min incubation at 72°C following amplification.

2.5. Cycle-sequencing

The PCR products were cloned in pBluescript plasmid (Stratagene) and were sequenced using the double-stranded DNA sequencing System (Gibco-BRL). Before performing cycle-sequencing, one set of degenerate primers were γ-^{32}P-labelled with T4 polynucleotide kinase. The cycle parameters were: 94°C for 4 min and 20 cycles at 94°C 30 s, 53°C for 30 s, 70°C for 60 s and 10 cycles at 94°C for 30 s, 70°C for 60 s.

2.6. Synthesis of the radioactive probe

The radioactive probe was synthetized by PCR amplification from a plasmid containing the *K. marxianus* fragment using the degenerate oligonucleotides as primers. The 20-μl reaction contained 100 pmol of each of the degenerate primers, incubation solution as described above in Section 2.4., dATP, dGTP, dTTP to a final concentration of 60 μM, 0.825 μM [α-^{32}P]dCTP and 0.1 ng of plasmid. The cycle parameters were as described under Section 2.4.

2.7. β-Galactosidase assays

The cells were transformed by both *MIG1*-containing plasmid and *SUC2-lacZ* reporter plasmid and grown in synthetic 2% glucose media. They were harvested in log phase prior to the β-galactosidase assay performed as described by Östling et al. [14]. Specific β-galactosidase activities presented in the text are mean values from five independent transformants for each plasmid.

3. Results and discussion

3.1. Isolation of a PCR probe

Sequence comparisons between the *S. cerevisiae* Mig1 and *A. nidulans* CREA proteins revealed that the two zinc fingers are highly conserved [20]. Two sets of degenerate oligonucleotides were designed from 6 identical residues located in each loop of the two fingers (Fig. 1). These primers were used to amplify genomic DNA from *K. marxianus* and *K. lactis*. In each case, amplification yielded a unique product of the same size (data not shown). Their deduced amino acid sequences turned out to be closely related (1 substitution only) and were very similar to those of ScMig1 (4 substitutions) and CREA (2 or 3 substitutions).

3.2. Cloning and sequence analysis of the KlMIG1 gene

The *K. marxianus* PCR fragment was shown by Southern blotting to hybridize to both *K. marxianus* and *K. lactis* genomic DNA (data not shown). It was therefore used for screening a *K. lactis* CBS 2359 genomic library established in the shuttle vector KEp6 [21]. One of the positive clones (named pGIKL2–3) was shown to contain the *KlMIG1* gene, using a PCR amplification test with the degenerate primers described above. Primers taken from the *K. lactis* PCR fragment were used to initiate sequencing of the entire gene on both strands.

The *KlMIG1* gene encodes a protein of 474 amino acids with a calculated molecular weight of 53 kDa (Fig. 2). Except for the DNA-binding domain (see below), the *K. lactis* Mig1 protein displays a relatively low overall level of similarity (55%) to those of *S. cerevisiae* and *A. nidulans*. A weak structural conservation outside the DNA-binding domain is also observed for other transcriptional regulators such as KlLac9, KlCpf1 and KlAbf1 [22–24]. Most of the conserved residues are concentrated in the the N-terminal zinc finger domain where 45/51 amino acids are identical. In the loops of the two fingers, the sequences HRLEHQT and DELTRH, from which the degenerate primers were designed, are strictly conserved. The second loop sequence is also present in the zinc finger domain of the mammalian Zif268 protein (Krox24) [25]. According to the three-dimensional structure of Zif268 [26] and to the model proposed by Lundin et al. for ScMig1 [4], residues 15, 18 and 21 of each finger (arrows, Fig. 1) are in contact with a base triplet in the GC-rich MIG1-binding site. These amino acids are the same in both Mig1 proteins (asterisks, Fig. 2) as well as in CREA (arrows, Fig. 1). The high conservation observed in the zinc finger region suggests that the rules governing Mig1-binding to its target DNA are the same in all fungi.

Outside of the DNA-binding domain, the sequences of KlMig1 and ScMig1 proteins largely diverge except in certain short motifs (Fig. 2, underlined). Thus, they share a well-conserved central sequence (13/16 identical amino acids) which contains potential sites for protein phosphorylation [27]. A second conserved region contains a KKSK motif which also seems to be a good candidate for phosphorylation. Two internal elements that mediate negative control of Mig1 activity in the absence of glucose have been mapped in ScMig1 [14]. However, these elements are distinct from the two conserved regions discussed above. The function of the latter there remains to be determined. Interestingly, the extreme C-terminal part of KlMig1 is also relatively well-conserved (7/13 identical residues). In *S. cerevisiae*, deletion analysis has shown that this small region constitutes an effector domain which is necessary and sufficient for mediating repression of the *SUC2* promoter [14]. This region contains repetitions of the LP dipeptide (3 in ScMig1 and 2 in KlMig1). The presence of a conserved arginine between the two dipeptides suggests that the adjacent serine could be phosphorylated [27]. It should be noticed that LP motifs are also present in the Rox1 repressor of *S. cerevisiae* [28], which represses hypoxic gene expression and is also

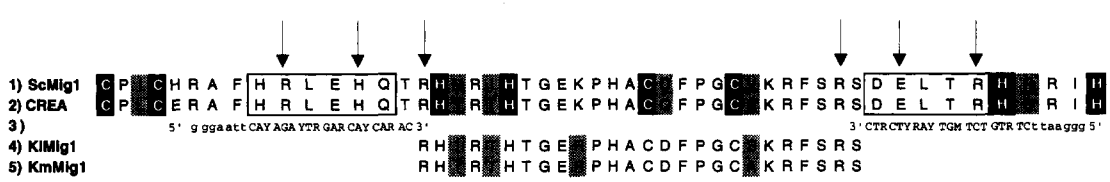

Fig. 1. Amino acid sequences of the zinc finger regions from *S. cerevisiae* Mig1 (line 1) and *A. nidulans* CREA protein (line 2). The cysteine and histidine residues of the C_2H_2 motifs are highlighted in black boxes and the divergent residues are stippled. Residues thought to be in contact with the GC-rich MIG1-binding site are marked by arrows. The 6 identical residues used to design the degenerate primers (line 3) are boxed (M = A or C; R = A or G; Y = C or T). Unpaired nucleotides providing *Eco*RI sites are shown in lower case. Lines 4 and 5 show the amino acid sequences of the PCR fragments from *K. lactis* and *K. marxianus*, respectively.

thought to recruit the Ssn6-Tup1 complex to its target promoters [29].

3.3. *KlMIG1 is functional in S. cerevisiae*

In view of the weak overall structural similarity between KlMig1 and ScMig1, it was of interest to investigate whether the two transcription factors could be functionally exchanged.

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GTTTGACCATATCACGGGTCTTTCTGCTGTAATACTTCAAGGCAAGCTTCCATATTATTT   60
TGGTTTTTGGTCTTGTTATTTGGTGACTGATACGAAAGGAATCTGGAACTTATAGCACCA  120
ATGACAGAGGCGATTATAGAGAAAAAAAATCATAAGAAGTCTATCAATGATCATGACAAG  180
M  T  E  A  I  I  E  K  K  N  H  K  K  S  I  N  D  H  D  K    20

GATGGACCAAGGCCTTACGTCTGTCCCATATGTCAAAGGGGATTCCATCGACTGGAACAT  240
D  G  P  R  P  Y  V  C  P  I  C  Q  R  G  F  H  R  L  E  H    40

CAGACTAGGCACATCAGAACACACACTGGGGAAAGACCGCATGCATGTGATTTCCCGGGA  300
Q  T  R  H  I  R  T  H  T  G  E  R  P  H  A  C  D  F  P  G    60

TGTTCAAAACGCTTTAGTAGAAGCGATGAACTAACAAGACATAGAAGGATACATGACAGT  360
C  S  K  R  F  S  R  S  D  E  L  T  R  H  R  R  T  H  D  S    80

GATAAACCAAAGGGGAAAAGAGGAAGGAAAAAGAAGAGTGAGACGATAGCTCGTGAAAAG  420
D  K  P  K  G  K  R  G  R  K  K  K  S  E  T  I  A  R  E  K   100

GAATTAGAATTGCAGCGGCAAAAACAACGAAACGCAAACGACTCTGCGGCTGTTGATTCT  480
E  L  E  L  Q  R  Q  K  Q  R  N  A  N  D  S  A  A  V  D  S   120

GCTGGTGGAACGAGCGCTAATGTCATAGAACCAAACCACAAACTTCTGAAATCCACTAAT  540
A  G  G  T  S  A  N  V  I  E  P  N  H  K  L  L  K  S  T  N   140

TCGATTAAACAAGATGGTTCAACATTTACTGAACCTCTGAAATCGTTGAGGTCGAAGCCA  600
S  I  K  Q  D  G  S  T  F  T  E  P  L  K  S  L  R  S  K  P   160

ATGTTTGATCTCGGTAGCGATGAATCGGATGAATGCGGTATATATAGTGTCCCACCTATT  660
M  F  D  L  G  S  D  E  S  D  E  C  G  I  Y  S  V  P  P  I   180

AGATCTCAGAATAATAGTGGTAACATAGACCTTCTGCTGAATGCTGCAAAATTTGAGTCT  720
R  S  Q  N  N  S  G  N  I  D  L  L  L  N  A  A  K  F  E  S   200

GACAAAGCCTCATCCTCTTTCAAATTTATTGATAAACTACCGTTGACTTCATCTTCATCC  780
D  K  A  S  S  S  F  K  F  I  D  K  L  P  L  T  S  S  S  S   220

TCTCCGTCACTTTCGTTTACATCTCATTCAATCAACAACAGCAGTAGCGGACTATTGTTA  840
S  P  S  L  S  F  T  S  H  S  I  N  N  S  S  S  G  L  L  L   240

CCAAGACCAGCTTCACGTGCTAAGCTTTCTGCTTTATCATCATTACAAAGAATGACACCC  900
P  R  P  A  S  R  A  K  L  S  A  L  S  S  L  Q  R  M  T  P   260

TTGTCTCAAAATTCAGAGTCATATAATCATTCGCAACAGAATCTAGTACATCTTCACCAT  960
L  S  Q  N  S  E  S  Y  N  H  S  Q  Q  N  L  V  H  L  H  H   280

CCCGCACCCAACCGACCATTGACCGAGTTTGTTGATAACGAGTATATAAGTAACGGTCTG 1020
P  A  P  N  R  P  L  T  E  F  V  D  N  E  Y  I  S  N  G  L   300

CCTAGAACCAGATCGTGGACAAATCTGTCGGAACAGCAATCACCATCGGGCTTCAGCTCC 1080
P  R  T  R  S  W  T  N  L  S  E  Q  Q  S  P  S  G  F  S  S   320

TCTGCACTTAACTCCAGATTCTCGTCATCCAATAGTCTCAACCAACTGATAGATCAACAT 1140
S  A  L  N  S  R  F  S  S  S  N  S  L  N  Q  L  I  D  Q  H   340

TCAAGAAATTCAAGTACTGTAAGCATATCTACTCTACTGAAGCAAGAAACCGTAATCTCA 1200
S  R  N  S  S  T  V  S  I  S  T  L  L  K  Q  E  T  V  I  S   360

CAAGATGAGGATATGAGTACAGAAGATGCATATGGCCGGCCACTTAAGAAATCAAAAGCC 1260
Q  D  E  D  M  S  T  E  D  A  Y  G  R  P  L  K  K  S  K  A   380

ATAATGCCCATCATGAGACCTAGTTCTACAATGCCACCAAGTTCCGGCTCAGCTACAGAA 1320
I  M  P  I  M  R  P  S  S  T  M  P  P  S  S  G  S  A  T  E   400

GGAGAATTTTATGATGAACTTCATTCAAGGCTTAGATCGATGGATCAACTGCCGGTAAGG 1380
G  E  F  Y  D  E  L  H  S  R  L  R  S  M  D  Q  L  P  V  R   420

AACAGTAAGGACGAAAAAGATTACTATTTCCAAAGTCATTTTTCAAGTTTACTGTGCACT 1440
N  S  K  D  E  K  D  Y  Y  F  Q  S  H  F  S  S  L  L  C  T   440

CCAACGCACAGTCCTCCACCGGAAGGATTGTTACCCAGTTTGAATCAGAATAAGCCAGTG 1500
P  T  H  S  P  P  P  E  G  L  L  P  S  L  N  Q  N  K  P  V   460

CAGTTGCCATCACTTCGAAGCTTAGACCTTTTACCACCAAAATAATGACTACTAATGAAA 1560
Q  L  P  S  L  R  S  L  D  L  L  P  P  K                    474

ATGGACAGAAATGTAAGCGCACCGTCTCTTCATCGATCAATGTATTATATTAACTCAAGA 1620
TATAAAACTTTCACGCATAATGAATATAGAATTTTACTAGCTCTTGTCCATCAATTATAT 1680
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Fig. 2. Sequence of the *KlMIG1* gene and its encoded protein (GenBank accession number Z50017). The N-terminal zinc finger domain is underlined. The central and C-terminal motifs that are conserved in ScMig1 are also underlined. Identities between ScMig1 and KlMig1 are circled. Amino acids thought to contact with DNA in ScMig1 [4, 26] are indicated by asterisks.

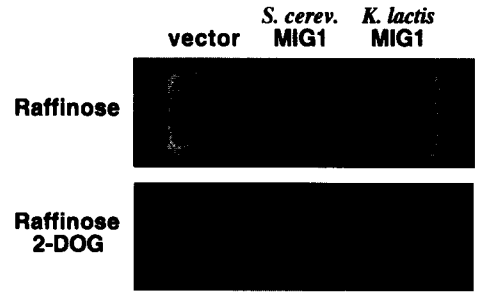

Fig. 3. Complementation of a *mig1* mutant of *S. cerevisiae* with the *K. lactis MIG1* gene. Cells were first grown on histidine-less 2% glucose media and then replicated to histidine-less 3% raffinose media. The first lane illustrates the results of transformation with the vector alone; the second and third lanes illustrate complementation with the *MIG1* genes from *S. cerevisae* and *K. lactis*, respectively.

We first introduced the *KlMIG1 gene* into a *mig1* mutant of *S. cerevisiae* which is able to grow on raffinose in the presence of 2-deoxyglucose due to the relief of *SUC2* gene repression [3]. As it can be seen in Fig. 3, heterologous complementation was effective since transformation of *S. cerevisiae* by the *K. lactis MIG1* gene prevented growth as efficiently as the *ScMIG1* gene. We also introduced the *K. lactis MIG1* gene into a *mig1* mutant of *S. cerevisiae* harboring a β-galactosidase reporter gene driven by the *SUC2* reporter. Again, repression was clearly achieved by KlMig1 since β-galactosidase levels were reduced to 1.49 ± 0.28 U as compared with 4.98 ± 0.48 U in the negative control and 0.86 ± 0.14 U in cells carrying the *ScMIG1* gene. Altogether, these data demonstrate that the *K. lactis* Mig1 protein, despite the evolutionary distance, can function in *S. cerevisiae*.

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