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A novel mechanism for target gene-specific SWI/SNF recruitment via the Snf2p N-terminus

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ABSTRACT

Chromatin-remodeling complexes regulate the expression of genes in all eukaryotic genomes. The SWI/SNF complex of *Saccharomyces cerevisiae* is recruited to its target promoters via interactions with selected transcription factors. Here, we show that the N-terminus of Snf2p, the chromatin remodeling core unit of the SWI/SNF complex, is essential for the expression of *VHT1*, the gene of the plasma membrane H^+/biotin symporter, and of *BIO5*, the gene of a 7-keto-8-aminopelargonic acid transporter, biotin biosynthetic precursor. chromatin immunoprecipitation (ChIP) analyses demonstrate that Vhr1p, the transcriptional regulator of *VHT1* and *BIO5* expression, is responsible for the targeting of Snf2p to the *VHT1* promoter at low biotin. We identified an Snf2p mutant, Snf2p-$R_{15}C$, that specifically abolishes the induction of *VHT1* and *BIO5* but not of other Snf2p-regulated genes, such as *GAL1*, *SUC2* or *INO1*. We present a novel mechanism of target gene-specific SWI/SNF recruitment via Vhr1p and a conserved N-terminal Snf2p domain.

INTRODUCTION

The Snf2p protein (synonym Swi2p) from baker's yeast (*Saccharomyces cerevisiae*) is the founding member of a huge family of nuclear regulators that are ubiquitous in eukaryotes, but found also in bacteria and archaea (1). The *SNF2* gene was originally identified in screens for *S. cerevisiae* mutants defective in the expression of the *HO* gene involved in mating type switching [SWI = switching defective (2)] or for mutants affected in the expression of the *SUC2* gene encoding the yeast invertase [SNF = sucrose non-fermenting (3)]. Snf2p family proteins are DNA-dependent ATPases that share a conserved region of helicase-related motifs (4). Most of these proteins act as ATP-dependent DNA translocases that can distort DNA and disrupt or remodel the structure of chromatin (RSC) that otherwise blocks the access of transcriptional activators or the basal transcriptional machinery to recognition sequences within target promoters (1).

In the cell, chromatin remodeling factors like Snf2p work in conjunction with other proteins, and the resulting complexes perform critical functions in the maintenance and expression of the genome (5–7). Baker's yeast has two SWI/SNF-type remodeling complexes, ySWI/SNF (yeast SWI/SNF complex) and RSC, that regulate different, largely non-overlapping sets of genes (6). Based on comparative expression analyses of yeast Δ*snf2* and Δ*swi1* mutants, it can be estimated that ySWI/SNF regulates roughly 6% of all *S. cerevisiae* genes (8). In contrast to RSC, ySWI/SNF is not essential for the viability of yeast cells (9). These all demonstrates that despite their common chromatin remodeling functions, these complexes are highly specific for different promoters. In human cells, which contain multiple SWI/SNF-like complexes, the distribution of regulatory functions between these complexes is even more elaborate (10).

A central question is, how the specificity of different SWI/SNF complexes for certain sets of promoters is achieved. *In vitro* analyses revealed that interaction of ySWI/SNF with the activation domain of Gcn4p, a transcriptional activator of amino acid biosynthetic genes, is necessary for recruitment of ySWI/SNF to Gcn4p-regulated promoters (11,12). Similarly, ySWI/SNF was shown to interact with the acidic activation domains of Swi5p known to recruit ySWI/SNF to the yeast *HO* promoter, or of Hap4p that regulates respiratory functions (12). Interaction of ySWI/SNF was even observed with the acidic activation domain of VP16, a transcriptional regulator of Herpes simplex virus (12,13), or with Gal4p-AH, a fusion of the Gal4p DNA-binding domain and a synthetic acidic helix (AH) (13). This led to the model that activation domain-mediated targeting of

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ySWI/SNF to promoters within nucleosome arrays is responsible for transcriptional activation.

In screens for ySWI/SNF components directly involved in this physical interaction with activation domains, the subunits Swi1p, Snf2p and Snf5p from *S. cerevisiae* could be characterized as interactors (14,15). Further analyses with *in vitro*-translated fragments of these three subunits identified two specific contact sites, one in the N-terminal domain of Snf5p and one in the second quarter of Swi1p (16,17).

No contact-specific interaction site could be determined for Snf2p from *S. cerevisiae*. However, interaction was demonstrated for one of two Snf2p orthologs from the SWI/SNF complex from man. These orthologs are human Brahma [hBrm (18)] and Brahma/SWI2-related gene 1 [BRG1 (19)]. BRG1 was shown to interact via its N-terminal helicase/SANT-associated (HAS) domain with the nuclear glucocorticoid receptor (20). This receptor targets the mammalian SWI/SNF complex to promoters containing glucocorticoid response elements (21).

SWI/SNF complexes can also repress transcription (5,10). The mammalian NuRD (nucleosome remodeling and histone deacetylation) complex was the first SWI/SNF-related complex shown to contain a histone deacetylase activity that is thought to be responsible for repression (22). In *S. cerevisiae*, ySWI/SNF is involved in the repression of the *SER3* gene that encodes a phosphoglycerate dehydrogenase catalyzing a step in serine biosynthesis (23). However, *SER3* repression depends on the enhanced expression of the non-coding *SRG1* gene (*SER3* regulatory gene 1) 5′ from *SER3* (24). ySWI/SNF is necessary for the induction of *SRG1* expression and, thus, only indirectly for the repression of *SER3* (25).

We previously characterized the product of the *S. cerevisiae VHT1* gene, Vitamin H Transporter 1, which encodes an H^+/biotin symporter of the yeast plasma membrane (26). *VHT1* is expressed only at low biotin concentrations. We demonstrated that the expression levels of *VHT1* and *BIO5* (Biotin biosynthesis 5), the gene of a plasma membrane transporter for the biotin biosynthetic precursor 7-keto-8-aminopelargonic acid (27), are regulated by Vhr1p [*VHT1* regulator 1 (28)] that binds to a vitamin H-responsive element (VHRE) in the *VHT1* and *BIO5* 5′-untranslated regions (28,29).

Here, we show that ySWI/SNF is essential for the induction of *VHT1* and *BIO5* expression, and that Snf2p is a central player in this regulation. An *snf2* mutant was identified in a complementation screen for *S. cerevisiae* mutants that had lost the capacity to express the gene of the green fluorescent protein (*GFP*) from the *VHT1* promoter. Unexpectedly, this *snf2* mutation turned out to specifically affect only the induction of *VHT1* and *BIO5*, but not the induction of *SUC2*, *INO1* and *GAL1* or the repression of *SER3*. Sequencing of the *snf2* mutant allele identified a point mutation near the N-terminus of Snf2p that converts a conserved arginine at position 15 into a cysteine ($R_{15}C$). Our data reveal a novel mechanism for target gene-specific ySWI/SNF recruitment in yeast via the N-terminus of Snf2p.

MATERIALS AND METHODS

Yeast strains and plasmids

Yeast strains used in this study are listed in Table 1. Strains FY3 and YBC3010 were gifts from Bradley Cairns (Salt Lake City, University of Utah, UT, USA).

For deletion of *VHR1* in YBC3010, the *Klyveromyces lactis URA3* disruption cassette of pUG72 (30) was amplified by PCR with the primers YIL056W-ko-5′ and YIL056W-ko-3′. Disruption of *VHR1* was confirmed by PCR with appropriate primers. The resulting strain was called MWY8003.

The mutant strain AMYmut153 (*snf2-$R_{15}C$*) was complemented with two plasmids from a YEp24 (2-μ plasmid)-based yeast genomic library (31) that contained *SNF2* as the only full-length open reading frame (ORF), including 3475 bp and 191 bp, or 3466 bp and 205 bp of upstream and downstream sequence, respectively. The *SNF2* gene, together with 2881 bp of upstream and 191 bp of downstream sequence, was isolated by restriction digest of one of the complementing plasmids with XmaI and was cloned into the XmaI-linearized single copy vector YCplac33 (32) yielding plasmid pMW814.

The mutation of the *snf2-$R_{15}C$* allele was introduced into pMW814 by site-directed mutagenesis with the primer ScSNF2+26f-mut153+T (Table 2) using the QuikChange Multi Kit (Stratagene, La Jolla, CA, USA). This primer also introduced a second, silent mutation into the *SNF2* ORF that destroyed a HincII to allow identification of mutated plasmids by restriction digest analysis. Sequence analysis of the resulting ORF confirmed that the encoded Snf2 protein differed only in the desired Arg to Cys exchange from the WT protein encoded by the original plasmid. The resulting plasmid was called pMW817.

A partial genomic sequence for an Snf2p-myc tag was amplified with the primers S3-SNF2 and S2-SNF2 from plasmid pYM6 (33) and inserted via homologous recombination into the *SNF2* locus of MWY760gc yielding strain MWY760gc-SNF2-myc. The 3′-end of this modified *SNF2* sequence was amplified by PCR (ScSNF2c+5047f and TRP primer), cloned into pJet1.2, sequenced and ligated via the BglII and XhoI restriction sites of this fragment into the BamHI/XhoI-cut yeast single copy vectors pMW814 or pMW817 that contain WT *SNF2* or mutated *snf2-$R_{15}C$* genomic sequences,

Table 1. Yeast strains used in this study

Strain	Genotype	Reference/Source
JS91.15-23	*MATα, his3-Δ1, leu2-3, trp1-289, ura3-52, can1*	(51)
JSYΔvht1	Isogenic to JS91.15-23; *vht1::HIS3*	(26)
MWY760gc	Isogenic to JS91.15-23; *vht1p::ADH1p*	(28)
AMYmut153	EMS mutant of MWY760gc; *snf2-153*	This study
BY4741	*MATa, his3-Δ1, leu2-Δ0, met15-Δ0, ura3-Δ0*	EUROSCARF
Y01586	Isogenic to BY4741; *snf2::kanMX4*	EUROSCARF
Y01250	Isogenic to BY4741; *swi3::kanMX4*	EUROSCARF
FY3	*MATa, ura3-52*	(52)
YBC3010	Isogenic to FY3; *SNF2-13myc::kanMX*	Bradley Cairns
MWY8003	Isogenic to YBC3010; *vhr1::klURA3*	This study

respectively. The resulting single copy plasmids with the BglII/XhoI insertions encoded Snf2p-myc or snf2p-R_{15}C-myc proteins.

Media, EMS mutagenesis, growth tests and induction tests

Ethyl methanesulfonate (EMS) mutagenesis and the complementation screening procedure were carried out as described (28). The stability of the identified mutants (= no revertants) and the absence of mutations in the *GFP* sequence were tested as published (28).

Rich medium and synthetic media with defined vitamin concentrations were prepared as described earlier (28). For growth tests on petri plates, yeast cells were suspended to an OD_{600} of 0.2 in ice cold water, diluted 1 : 8, 1 : 64 and 1 : 512, and 3 µl of each dilution were spotted onto the agar. For the growth tests with defined biotin concentrations, 1% agarose was used instead of agar agar. Media for induction tests of relevant genes were prepared as described elsewhere (28,34–36). Where rich medium could not be used for the induction tests (e.g. maintenance of plasmids), synthetic medium with 0.67% yeast nitrogen base and 2% casamino acids (Difco, Augsburg, Germany) was used. For the induction of *GAL1*, *SUC2* or *INO1*, yeasts were grown for one generation time in the respective medium. Induction of *VHT1* was performed as described (28).

Western blot analyses

Western blots were made after 8% sodium dodecylsulfate (SDS)–polyacrylamide gel electrophoresis with a monoclonal primary antibody against myc (9E10; Sigma, Taufkirchen, Germany) and a polyclonal rabbit antibody against purified *S. cerevisiae* alcohol dehydrogenase (gift from Prof. Christian Koch, University of Erlangen-Nuremberg, Germany). Secondary horse-radish peroxidase coupled anti-mouse antibody and anti-rabbit antibody were from Sigma (Taufkirchen, Germany). Antibody-binding was visualized with the ECL Lumilight-Kit from Roche (Mannheim, Germany).

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) was performed essentially as described (37) after a protocol adapted from (38) and (39). Antibodies used for ChIP assays were anti-myc 9E11 (Dianova, Hamburg, Germany) and αGFP MAB3580 (Chemicon/Millipore, Schwalbach, Germany). Dynabeads Protein G (Dynal/Invitrogen, Karlsruhe, Germany) were used as matrix. Primers used for amplification were ScVHT1g-361f and ScVHT1g-144r for the *VHT1* promoter, and ScACT1g+611f and ScACT1g+813r for normalization to the *ACT1* ORF. Real-time PCR was carried out as described (28).

RNA isolation and real-time reverse transcription PCR

RNA isolation and RT-PCRs were performed as described earlier (28). Oligonucleotides used for the amplification were ScACT1g-4f and ScACT1g+498r for *ACT1*, ScVHT1g+607f and ScVHT1g+808r for *VHT1*, ScBIO5g+1040f and ScBIO5g+1244r for *BIO5*, YIL056Wg+1411f and YIL056Wg+1617r for *VHR1*, ScSUC2g-1f and ScSUC2g+207r for *SUC2*, ScGAL1c+1379 and ScGAL1c+1576r for *GAL1*, ScINO1c+1316f and ScINO1c+1531r for *INO1* and ScSER3c+1084f and ScSER3c+1286r for *SER3*.

RESULTS

Identification of Snf2p as a factor involved in *VHT1* induction at low biotin concentrations

In a previous study, we identified the *S. cerevisiae* transcriptional regulator Vhr1p, the prototype of a new family of transcription factors, and showed that it is essential for induction of *VHT1* and *BIO5* at low biotin concentrations (28). *VHT1* and *BIO5* encode plasma membrane-localized transporters for biotin (vitamin H; Vht1p) or for its biosynthetic precursor 7-keto-8-aminopelargonic acid (Bio5p). The screen was performed with a strain (MWY760gc) that expressed *VHT1* under the control of the constitutive *ADH1* promoter allowing growth on low and high biotin. In addition, MWY760gc harbored a plasmid with a *VHT1* promoter (p*VHT1*)::*GFP* reporter cassette that drove expression of *GFP* only on low biotin (28). For the identification of *VHR1*, 150 000 EMS-mutagenized cells were analyzed.

To identify additional components of the biotin-dependent signaling cascade, we performed a new EMS mutagenesis on MWY760gc cells and screened additional 600 000 clones for loss of GFP fluorescence on low biotin. Fifteen newly obtained mutants were transformed with an *S. cerevisiae* genomic library (31) and a minimum of 200 000 transformants of each line was screened for recovery GFP fluorescence on low biotin. With 10 lines the complementation was successful, and for 8 lines the complementing sequences could be determined and assigned to complementation groups. The largest complementation group with 6 lines contained fragments carrying the *VHR1* gene (data not shown) confirming our previous results (28). One line was complemented by the *ECM1* gene (40) encoding a poorly characterized protein involved in cell wall biosynthesis (data not shown). This gene was not further analyzed. The last line, AMYmut153, was complemented by two different library inserts, each containing *SNF2* as the only full-length gene (Figure 1A).

To independently prove or disprove the identity of *SNF2* as the complementing gene, the *SNF2* coding sequence plus 2881 bp upstream and 191 bp downstream sequences was cloned into the single copy vector YCplac33 (32). The resulting plasmid, pMW814, was used to transform AMYmut153. GFP fluorescence was recovered only in the pMW814-transformed strain but not in the vector control (Figure 1A), which confirmed the importance of Snf2p for *VHT1* expression. We also analyzed expression of *VHT1* in the Δ*snf2* deletion mutant Y01586 (EUROSCARF, Frankfurt/Main, Germany) by quantitative RT-PCR (qRT-PCR; Figure 1B). In agreement with the previous data, the Δ*snf2* strain failed to induce *VHT1* under low biotin conditions. We finally tested if a mutation in another subunit of the ySWI/SNF complex will also abolish *VHT1* induction. We, therefore, analyzed *VHT1*

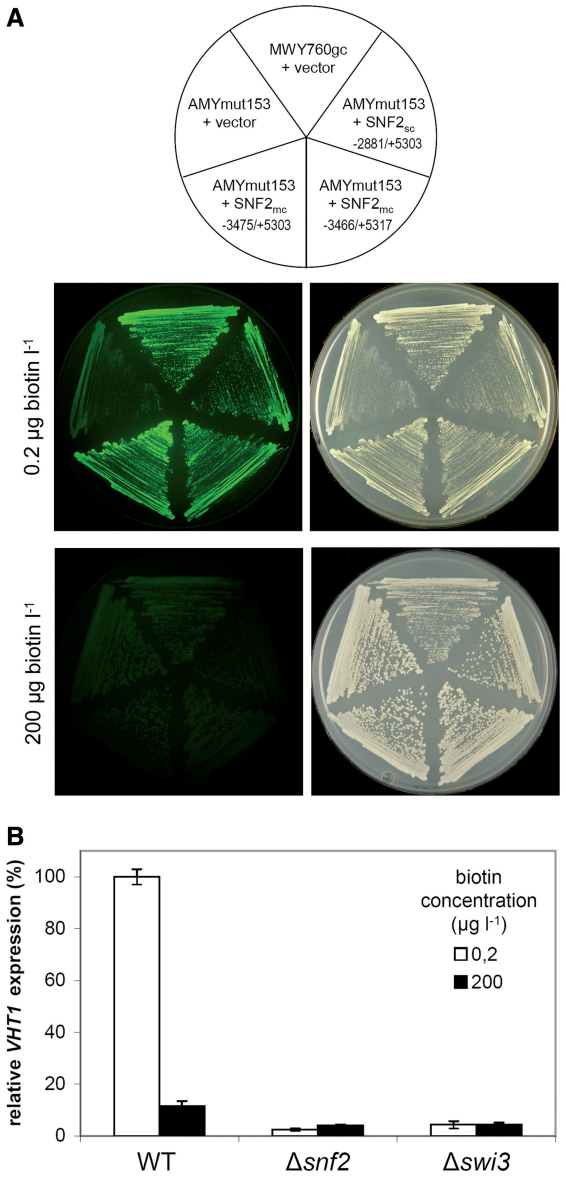

Figure 1. Identification of the ySWI/SNF complex as an essential component of *VHT1* induction. (**A**) *SNF2* complements the non-fluorescence phenotype of the EMS mutant AMYmut153. Images show the GFP fluorescence resulting from a p*VHT1*::*GFP* construct in the WT (MWY760gc) or in the AMYmut153 mutant. Strains were complemented with one of the *SNF2*-containing multicopy (mc) plasmids identified in the complementation screen or with a single copy (sc) plasmid (pMW814) harboring *SNF2*. Vector controls (A) carried the plasmid YEp24. Numbers show insert lengths relative to the *SNF2* start ATG. Plates were photographed in white light and under GFP excitation light. (**B**) Real-time RT-PCR analysis of *VHT1* mRNA levels in the WT and in mutants lacking subunits of the ySWI/SNF complex (Δ*snf2* or Δ*swi3*). RT-PCRs were performed with total RNA from yeasts grown on SD medium containing the indicated biotin concentrations. Results were standardized to *ACT1* mRNA levels ($n = 3$; ± SE).

expression in the Δ*swi3* mutant Y01250 (EUROSCARF) on low and high extracellular biotin by qRT-PCR (Figure 1B). In fact, also the Δ*swi3* mutant was unable to induce *VHT1* under low biotin conditions, underlining the essential role of the ySWI/SNF complex for induction of *VHT1*.

A single amino acid exchange in the N-terminus of Snf2p is responsible for the lack of *VHT1* induction

When we sequenced the *snf2* mutant allele of the EMS-mutant strain, AMYmut153 from −830 bp upstream from the start-ATG to 52 bp downstream of the stop-codon, we found a single point mutation in the codon for the arginine residue at position 15 in Snf2p. This mutation changed CGC into TGC, which replaced the arginine at position 15 by a cysteine yielding snf2p-R_{15}C. A comparison of the Snf2p sequences from different fungi demonstrated that this amino acid is highly conserved in Snf2 proteins from other species of the order Saccharomycetales (Figure 2A). Occasionally, this arginine can be replaced by a lysine residue (see the sequence of the *Candida dubliniensis* Snf2 protein in Figure 2A).

Sequence alignments revealed that this arginine is embedded in a conserved domain of about 50 amino acids at the very N-terminus of these Snf2 proteins (Figure 2A). This conserved N-terminal domain is found exclusively in yeast Snf2 proteins, and is followed by a 100–250 amino acid region of low sequence similarity. To find possibly conserved structural motifs within this region, we performed *in silico* analyses on the first 100 amino acid of *S. cerevisiae* Snf2p and of 9 other Snf2 proteins with the Protein Homology/analogY Recognition Engine [PHYRE; http://www.sbg.bio.ic.ac.uk/phyre/ (41); output files are presented in Supplementary Figure S1]. In fact, all analyses revealed an identical structure for each of these sequences (Figure 2B). An α-helix of about 17 amino acids (α1) is predicted to start 6–10 amino acids downstream from the start methionine, and a short stretch of 6–7 disordered residues separates this first helix from a second, slightly longer α-helix (α2) of 18–25 amino acids. This α2-helix is followed by a longer disordered region (Figure 2B and Supplementary Figure S1).

The helices and the disordered sequences are predicted with maximal probability. In all predictions, the arginine residue modified in our EMS mutant and the conserved arginine or lysine residues in the other Snf2 proteins are located in the first half of the α1-helix. Based on the functional characterization of the snf2p-R_{15}C protein (see below), we refer to this conserved structural motif in Snf2p as EVA (expression of *VHT1* activating) domain.

To exclude the possibility that the observed *snf2-R_{15}C* mutation results in a reduced amount of snf2p-R_{15}C protein, e.g. as a result of reduced stability of the mutant protein, we compared the amounts of Snf2p and snf2p-R_{15}C proteins in yeast cells. To this end, we generated single copy plasmids encoding modified Snf2p and snf2p-R_{15}C proteins with C-terminal myc tags (Snf2p-myc and snf2p-R_{15}C-myc) and expressed these constructs in the Δ*snf2* mutant strain Y01586. The amounts of Snf2p-myc and snf2p-R_{15}C-myc were compared on western blots treated with anti-myc antiserum (anti-myc-AB). Figure 2C shows that the amount of snf2p-R_{15}C-myc proteins is not decreased compared with the Snf2p-myc control.

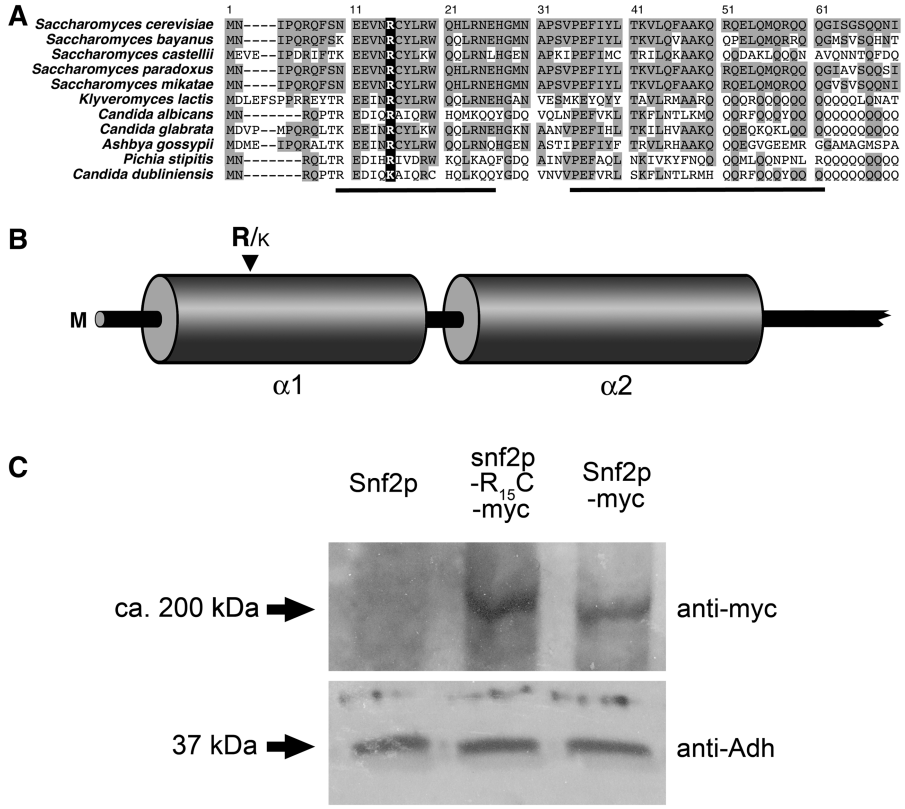

Figure 2. Alignment of N-terminal Snf2 protein sequences and comparative expression analyses. (**A**) The first 70 amino acids of *S. cerevisiae* Snf2p and the corresponding residues of Snf2 proteins from other members of the order Saccharomycetales. Amino acids identical in all proteins are highlighted. The arginine at position 15 in Snf2p is highlighted in black, predicted α-helices are underlined. (**B**) Model of the predicted secondary structure for Snf2p EVA domains. The position of the conserved arginine/lysine is indicated. (**C**) Western blot analysis of the relative amount of Snf2p-myc and snf2p-R_{15}C-myc in extracts of cells expressing the corresponding genes. An extract of cells expressing *SNF2* without a C-terminal fusion is shown to demonstrate the specificity of the anti-myc antiserum (anti-myc). Each lane was loaded with 5 µg of protein extract. Signals obtained from the same blot with an anti-Adh-antiserum (anti-Adh) are shown as loading controls.

The ySWI/SNF complex is recruited to p*VHT1* under inducing conditions

To test, if the observed Snf2p mutation reduces the expression of *VHT1* indirectly by affecting the expression of *VHR1*, the gene for the transcriptional regulator of *VHT1*, we compared *VHR1* mRNA levels in the Δ*snf2* strain Y01586 and in the corresponding wild type (WT) strain BY4741 (EUROSCARF) on low biotin. qRT-PCR reactions revealed no differences in the expression of *VHR1* (Figure 3) suggesting that the *snf2-R_{15}C* mutation affects *VHT1* expression directly, and that WT ySWI/SNF may bind directly to p*VHT1*.

We tested this hypothesis in ChIP analyses with strain YBC3010 that contains a myc-tagged version of Snf2p (Snf2p-myc). We assayed binding of Snf2p-myc to the vitamin H-responsive element (VHRE) relative to its binding to the *ACT1* ORF. We could confirm that Snf2p-myc is present at the VHRE at low, *VHT1*-inducing biotin concentrations (Figure 4). The signal obtained at non-inducing, high biotin concentrations was significantly lower and comparable with the signals obtained in two negative controls (Snf2p with no myc-tag or Snf2p-myc with a control antiserum against GFP, αGFP; Figure 4A). We also assayed binding of Snf2p-myc in a Δ*vhr1* deletion strain. In this strain, Snf2p-myc was not recruited to the VHRE, even when the cells were grown on low biotin. In summary, these data demonstrate (i) that Snf2p occupies the p*VHT1*, (ii) that this recruitment occurs at or near the VHRE element, (iii) that the presence of Vhr1p is essential to recruit Snf2p to the VHRE and (iv) that Vhr1p targets Snf2p to the *VHT1* promoter only at low biotin concentrations, i.e. under conditions, when *VHT1* expression is induced.

snf2p-R_{15}C cannot occupy p*VHT1*

The finding that Snf2p occupies the p*VHT1* (Figure 4A) suggested that this recruitment of Snf2p might be specifically affected by the mutation in the snf2p-R_{15}C protein. To test this hypothesis, we performed additional ChIP analyses with three yeast strains expressing *SNF2*, *SNF2-myc* or *snf2-R_{15}C-myc* in the background of the Δ*snf2* mutant strain Y01586 at low, *VHT1*-inducing biotin concentrations. Figure 4B demonstrates that as shown in Figure 4A Snf2p-myc occupies the p*VHT1*. Most importantly, however, Figure 4B also demonstrates that snf2p-R_{15}C is not recruited to the VHRE. Together with the data shown in Figure 4A, this result shows that snf2p-R_{15}C fails to induce *VHT1* expression, because its

$R_{15}C$ mutation abolishes the Vhr1p-mediated recruitment of Snf2p to the VHRE element in p*VHT1*.

ySWI/SNF mutants cannot grow on low biotin concentrations due to the lack of *VHT1* expression

Based on the data presented so far (Figures 1B, 3 and 4), we expected that *snf2* mutants should be severely affected in their capacity to grow on low biotin. We, therefore, compared the growth of the Δ*snf2* mutant, of the corresponding *SNF2* WT and of a Δ*vht1* deletion mutant [JSYΔvht1 (26)] on SD media with decreasing biotin concentrations. As expected, the Δ*vht1* mutant did grow only on high biotin (2 mg biotin l^{-1}; Figure 5). Also the Δ*snf2* cells failed to grow on medium with low biotin (0.02 μg biotin l^{-1}; Figure 5). The growth difference between Δ*vht1* and Δ*snf2* cells on medium biotin concentrations (200 μg biotin l^{-1}) results from a weak basal activity of p*VHT1* in Δ*snf2* cells and the complete absence of Vht1p activity in Δ*vht1* cells.

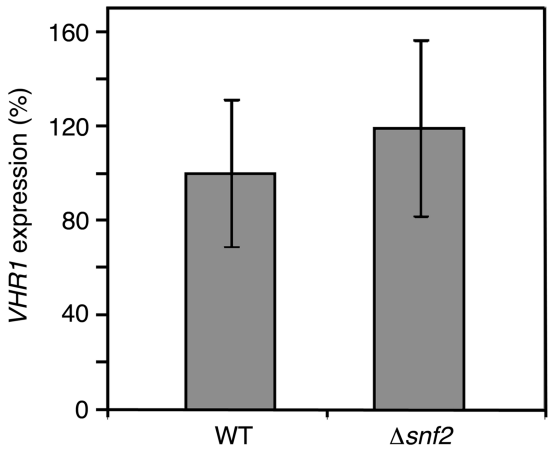

Figure 3. qRT-PCR of *VHR1* mRNA levels. *VHR1* mRNA levels were determined in the Δ*snf2* deletion mutant and in the corresponding WT on low biotin medium. Results were standardized to *ACT1* mRNA ($n = 3$; ± SE).

At high and medium biotin concentrations, the Δ*snf2* cells grow slower than the WT (Figure 5), a well-known behavior of ySWI/SNF mutants on glucose media (42). Transformation of the Δ*snf2* mutant with the pVHT1oe plasmid that expresses *VHT1* under the control of the constitutive *ADH1* promoter (26) complemented only the growth defect on low biotin, but not the slower growth of the Δ*snf2* mutant at higher biotin concentrations (Figure 5). The same results were obtained in experiments performed with the Δ*swi3* deletion mutant Y01250 (not shown). In summary, these results confirmed that mutations in ySWI/SNF subunits result in a lack of biotin-dependent *VHT1* induction (Figure 1B) and consequently in a reduced capacity to grow on low biotin (Figure 5).

The *snf2-$R_{15}C$* mutant has an unusual phenotype

Compared with WT cells, Δ*snf2* mutants exhibit slow growth on glucose [Figure 5 and (42,43)], fail to grow on galactose and raffinose (42,43), and are auxotrophs for inositol (44). This was re-confirmed in comparative growth analyses on media with galactose (YPGal) or raffinose (YPRaf) as sole carbon source, on medium w/o inositol (SD w/o inositol), or on glucose-containing yeast peptone dextrose (YPD) medium (Figure 6). Surprisingly, however, the *snf2-$R_{15}C$* mutant did not

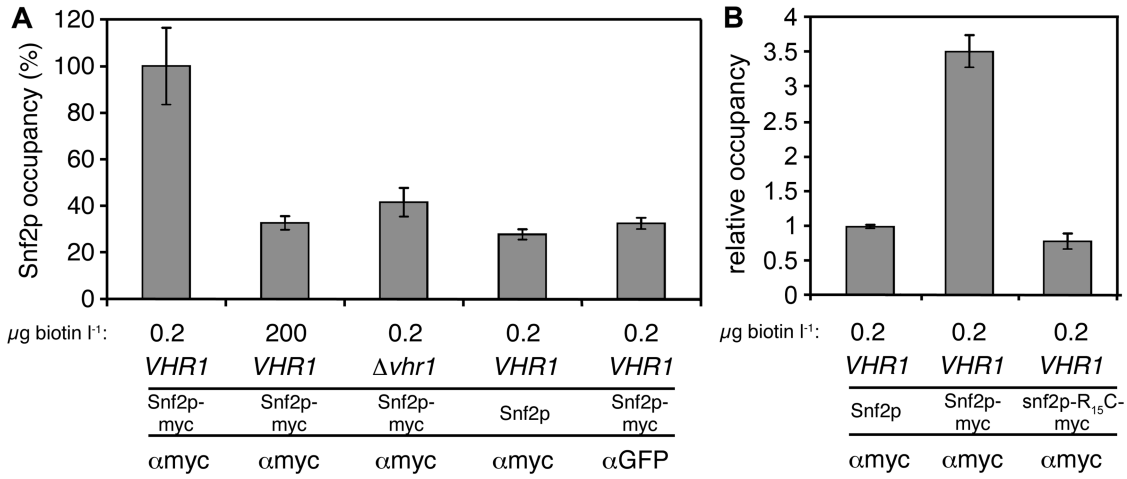

Figure 4. ChIP analyses with Snf2p-myc and snf2p-R_{15}C-myc. (**A**) ChIPs of Snf2p-myc were performed in a *VHR1* WT (YBC3010), a Δ*vhr1* mutant (MWY8003) and in a *VHR1* control strain (FY3) expressing untagged *SNF2*. ChIP was also performed in the *SNF2-myc*, *VHR1* strain (YBC3010) with a control antibody (αGFP). Yeast cells were grown on SD medium containing the indicated biotin concentrations. Binding of Snf2p-myc to the *VHT1* promoter was assayed relative to its binding at the *ACT1* ORF. Input control and immunoprecipitated DNA (IP) were amplified with primers spanning a region containing the *VHT1* promoter vitamin H-responsive element (VHRE). Snf2p occupancy was calculated as ratio of the *VHT1* signal in the IP-to-input samples and normalized for the corresponding ratio calculated for *ACT1*. The mean value for Snf2p-myc under inducing conditions was set to 100% ($n = 3$; ±SE). (**B**) ChIPs of Snf2p (control), Snf2p-myc and snf2p-R_{15}C-myc were performed in the Δ*snf2* mutant strain Y01586 expressing *SNF2* (control), *SNF2-myc* or *snf2-R_{15}C-myc* from a single copy plasmid under the control of the *SNF2* promoter. Binding of Snf2p-myc and snf2p-R_{15}C-myc to the *VHT1* promoter was assayed as described in (A). The mean signal obtained with the control strain expressing untagged *SNF2* was set to 1 ($n = 3$; ±SE).

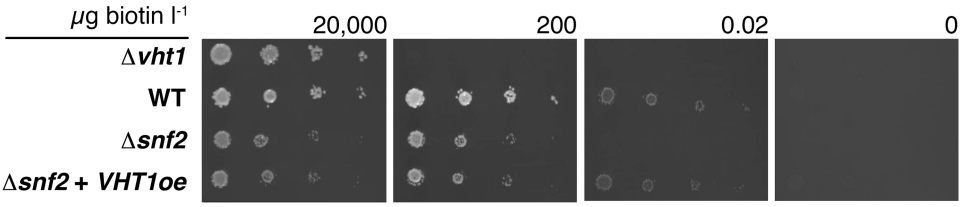

Figure 5. Growth test on SD medium with varying biotin concentrations. The Δ*snf2* mutant was transformed with plasmid pVHT1oe, expressing *VHT1* from the constitutive *ADH1* promoter (Δ*snf2* + VHT1oe). To allow growth on the same medium, the deletion mutants Δ*vht1* and Δ*snf2* and the WT were transformed with the empty vector NEV-E. Cells were spotted on SD medium containing the indicted biotin concentrations after serial 1:8 dilutions, grown for 3 days and photographed.

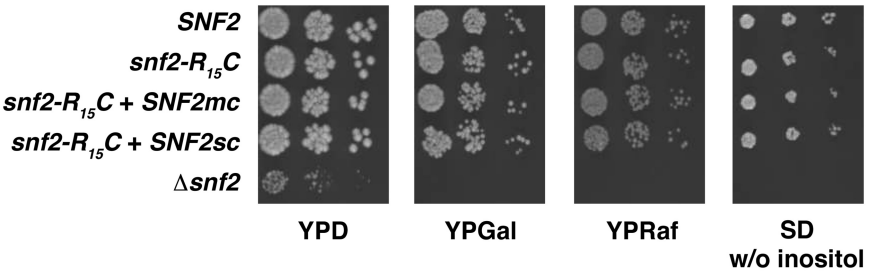

Figure 6. Growth test to assay phenotypes of ySWI/SNF mutants. Yeast strains were grown on the indicated media to assay the following phenotypes: slow growth (YPD), inability to use the sugars galactose or raffinose as carbon sources (YPGal and YPRaf), and inositol auxotrophy (SD w/o inositol). The *SNF2* WT (top) and the Δ*snf2* mutant (bottom) were used as controls to assay the growth behavior of the *snf2-R*$_{15}$*C* mutant. In addition, the effects of *SNF2* expression from one of the complementing multicopy plasmids (*snf2-R*$_{15}$*C* + *SNF2mc*; Figure 1A) or from an *SNF2*-containing single copy plasmid (*snf2-R*$_{15}$*C* + *SNF2sc*) were analyzed. To allow growth on the same medium, control strains were transformed with the empty plasmid YEp24. Cells were spotted on plates after serial 1:8 dilutions.

show any of these phenotypes and behaved like the WT on all media (Figure 6). Moreover, this growth could not be further improved by expressing WT *SNF2* from one of the multicopy plasmids (*snf2-R*$_{15}$*C* + *SNF2mc*) identified in the complementation screening (Figure 1A) or from the single copy plasmid (*snf2-R*$_{15}$*C* + *SNF2sc*) used to express *SNF2* in the AMYmut153 strain (Figure 1A). This demonstrated that the *snf2-R*$_{15}$*C* mutation does not exhibit any of the previously published growth phenotypes of a Δ*snf2* deletion mutant. It rather suggested that the *snf2-R*$_{15}$*C* mutation is hypomorphic allele that specifically affects the regulation by Vhr1p and the targeting of ySWI/SNF to the VHRE.

snf2p-R$_{15}$C fails to induce *VHT1* and *BIO5*, but mediates WT-like expression of the ySWI/SNF-dependent genes *SUC2*, *GAL1*, *INO1* and *SER3*

The unexpected WT-like growth of the *snf2-R*$_{15}$*C* mutant on YPGal and YPRaf medium or on SD medium w/o inositol pointed toward a normal, WT-like expression of the responsible ySWI/SNF target genes, *GAL1*, *SUC2* and *INO1*. We tested the expression of these genes by RT-PCR in the *SNF2* WT, the Δ*snf2* strain and the *snf2-R*$_{15}$*C* mutant that were grown on media inducing the expression of *SUC2* (YPRaf), *GAL1* (YPGal) or *INO1* (SD medium w/o inositol). Moreover, we analyzed the expression of *SER3*, a gene of the serine biosynthetic pathway known to be de-repressed in Δ*snf2* mutants on rich medium (25). As expected, the mRNA levels of all genes were comparable in the *SNF2* WT strain and in the *snf2-R*$_{15}$*C* mutant (Figure 7), whereas the Δ*snf2* deletion mutant failed to induce expression of *GAL1*, *SUC2* and *INO1*, and did not repress *SER3*.

We could not include analyses of *VHT1* expression in Figure 7, as the *snf2-R*$_{15}$*C* mutant used in these analyses (AMYmut153; see Figure 1) expressed *VHT1* from the constitutive *ADH1* promoter. For a direct comparison of the *snf2-R*$_{15}$*C*-dependent induction of *VHT1* with the induction/repression of other ySWI/SNF target genes in WT and Δ*snf2* cells, we introduced the *snf2-R*$_{15}$*C* mutation into the single copy plasmid pMW814 used in Figure 1 by site-directed mutagenesis. We next transformed the Δ*snf2* strain Y01586 either with this plasmid to obtain the *snf2-R*$_{15}$*C* genotype, with the *SNF2* WT plasmid to regain the WT gene, or with the empty vector to maintain the Δ*snf2* genotype. To study the expression of *VHT1*, *GAL1* and *SER3* by quantitative real-time RT-PCR, these three strains were grown either on SD medium with low biotin (0.2 µg l^{-1}; Figure 8A), on galactose medium (Figure 8C), or on CAA medium (Figure 8D). Moreover, we included analyses of *BIO5* expression, another gene induced by the Vhr1p transcription factor on low biotin [Figure 8B and ref. (28)].

As expected, expression of *VHT1* was very high in the *SNF2*-complemented Δ*snf2* mutant, but negligible in the vector control and in the *snf2-R*$_{15}$*C* complemented strain (Figure 8A), which confirmed our previous experiments (Figures 1, 4 and 5). A very similar result was obtained for *BIO5* (Figure 8B), which demonstrated that the *snf2-R*$_{15}$*C* mutation affects the expression of other Vhr1p-regulated genes in the same way as *VHT1*. In contrast, and in agreement with the data shown in Figures 6 and 7, *snf2-R*$_{15}$*C* and Δ*snf2* differed in

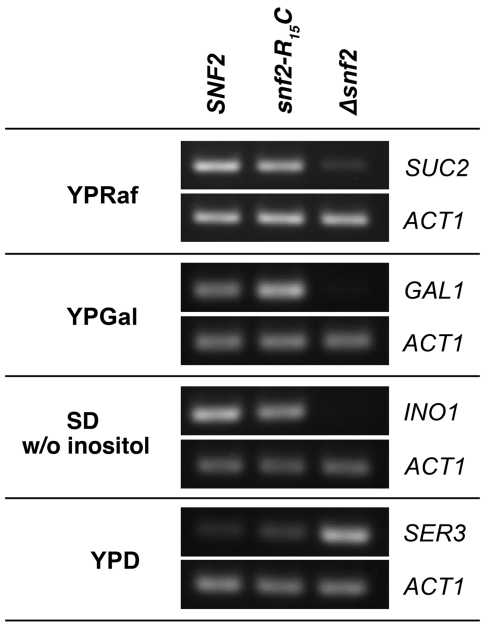

Figure 7. Comparative qRT-PCR analysis of the expression of typical ySWI/SNF target genes. RT-PCR analysis of mRNA levels of *SUC2*, *GAL1* and *INO1* under inducing conditions, and of *SER3* under repressing conditions. The *snf2-R*$_{15}$*C* mutant strain, the Δ*snf2* deletion mutant and the WT strain were first grown in YPD medium, cultures were split and incubated in the indicated media.

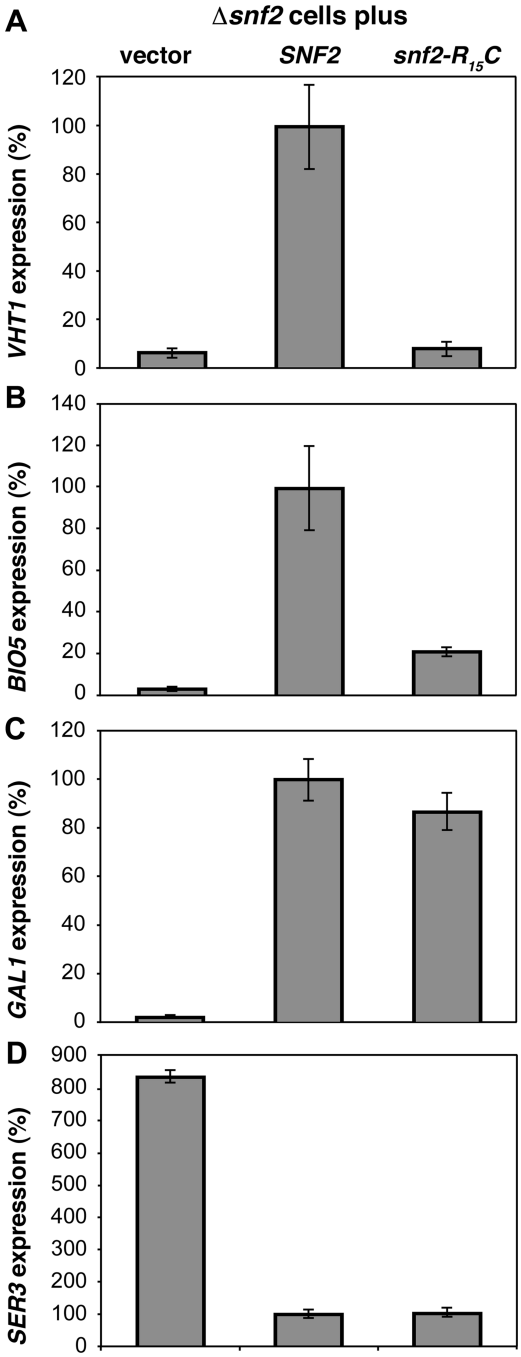

Figure 8. qRT-PCR analysis of mRNA levels of *VHT1*, *BIO5* and *GAL1* under inducing conditions and of *SER3* under repressing conditions. The Δ*snf2* deletion mutant was transformed with the single copy plasmids harboring the *SNF2* gene, with the *snf2-R*$_{15}$*C* mutant allele, or with the YEp24 vector (maintaining the Δ*snf2* genotype). After growth in CAA liquid medium, cultures were split and incubated in the following media: (**A**) and (**B**) in low biotin medium, (**C**) in CAA medium supplemented with 2% galactose, (**D**) CAA medium with 2% glucose. Results were standardized to *ACT1* mRNA levels; expression levels in *SNF2* complemented cells were set to 100% ($n = 3$; ±SE).

their capacity to induce *GAL1* on galactose medium (Figure 8C), and in their capacity to repress *SER3* (Figure 8D). The expression of both genes was identical in *snf2-R*$_{15}$*C* complemented and *SNF2* complemented cells. Again this confirmed that the *snf2p-R*$_{15}$*C* allele encodes a protein capable to regulate well-known ySWI/SNF target genes, such as *GAL1* or *SER3*. In contrast, its capacity to induce Vhr1p-regulated genes, such as *VHT1* and *BIO5*, is completely lost.

DISCUSSION

The nucleosome remodeling ATPase Snf2p represents the central catalytic subunit of the ySWI/SNF complex that regulates a large number of *S. cerevisiae* genes (6,7,45). In a complementation screen for transcriptional regulators of *VHT1*, the gene of the biotin/H^+ symporter, we identified a novel *snf2* mutant allele (Figure 1A). We verified the importance of Snf2p and the ySWI/SNF complex for *VHT1* induction in additional analyses of Δ*snf2 and* Δ*swi3* mutants (Figure 1B) and characterized *VHT1* and *BIO5* as previously unidentified target genes of ySWI/SNF. They were not identified in former large-scale searches for ySWI/SNF target genes (5), as *VHT1* and *BIO5* stay repressed under the high biotin conditions applied in these experiments.

The R_{15}C mutation in the newly identified snf2p mutant allele is located in the first of two α-helices in the structurally conserved EVA domain found in the Snf2p proteins of all Saccharomycetes (Figure 2A and Supplementary Figure S1). Occasionally, e.g. in Snf2p of *Debaryomyces hansenii* (accession number XP_461680) or *Candida dubliniensis* (accession number CAX43468), this conserved arginine can be replaced by a lysine residue. Both, this conserved basic residue and the EVA domain, are absent from other chromatin remodeling ATPases of *S. cerevisiae*

Table 2. Oligonucleotides used in this study

Oligonucleotide	Sequence
ScACT1g-4f	AACAATGGATTCTGAGGTTGCTGC
ScACT1g+498r	TCTCTTGGATTGAGCTTCATCACC
ScACT1g+611f	CCCTGTTCTTTTGACTGAAGC
ScACT1g+813r	AACCAGCGTAAATTGGAACG
ScBIO5g+1040f	AAACGGCAGTCGTTGAGTCT
ScBIO5g+1244r	TTTCCCAAGGAACCCTCTTT
ScGAL1c+1379	CATATGGTTCCCGTTTGACC
ScGAL1c+1576r	ATAGACAGCTGCCCAATGCT
ScINO1c+1316f	CTTTACTGGCTACGCCCTTG
ScINO1c+1531r	CTAAGGCGGTTCTTTGCTTG
ScSER3c+1084f	GAAGTGGCTACCGCATTGTC
ScSER3c+1286r	TCGGAAAATTGCTTTTCGAT
ScSNF2+26f-mut153+T	GCAACGAAGAGGTCAATTG CTGCTATTTAAGATGG
ScSNF2c+5047f	ACTTCAAGCGTGGCTGAATC
ScSUC2g-1f	GATGCTTTTGCAAGCTTTCCTT
ScSUC2g+207r	ACCCCATACGGTGTCATTTG
ScVHT1g+607f	GGCTGGACTTGGTTCACATT
ScVHT1g+808r	CTGAAGTCACGGAACCCAAT
ScVHT1g-361f	TGATTGCCTCCATTGCTACA
ScVHT1g-144r	GCCTTGCATCTCATATTTCG
S2-SNF2	GTCTACGTATAAACGAATAAGT ACTTATATTGCTTTAGGAAG GTAATCGATGAATTCGAGCTCG
S3-SNF2	TCTTTCACAGATGAAGCGGAC TCGAGCATGACAGAAGCGAG TGTACGTACGCTGCAGGTCGAC
TRP primer	GCTATTCATCCAGCAGGCCTC
YIL056Wg+1411f	CCCACGGTGTCTGAAGTTTT
YIL056Wg+1617r	GGGCACTGGTATTTTTGCAT
YIL056W-ko-5′	TTCTACCTATTCTTCTTCGTTGC TTATATCCTATACACATCAG CTGAAGCTTCGTACGC
YIL056W-ko-3′	AATGTGTATTAGGCAGTTATGTA TGTAGACAATTTTGTTTGC ATAGGCCACTAGTGGATCTG

including the less closely related Isw1p, Isw2p, Chd1p, Ino80p and Swr1p proteins, and also the closely related Sth1p protein of the RSC complex (46). This points toward a conserved function of Snf2p N-termini.

Binding of Snf2p to the *VHT1* promoter depends on the presence of Vhr1p

ChIP analyses confirmed the presence of Snf2p at the *VHT1* promoter in a 200-bp region (Figure 4) containing the VHRE essential for biotin-dependent *VHT1* induction (28,29). This binding of Snf2p was detected only under inducing conditions, i.e. at low biotin. The binding of Snf2p also depended strictly on the presence of Vhr1p, the transcriptional regulator. No binding was detected in a Δ*vhr1* deletion mutant (Figure 4) that is unable to induce *VHT1* (28). This demonstrated that Vhr1p is essential for Snf2p recruitment to the *VHT1* promoter. It is, however, not yet possible to distinguish whether there is a direct or indirect interaction between Vhr1p and the N-terminus of Snf2p.

The *snf2-R_{15}C* mutant represents a hypomorphic allele

Surprisingly, the *snf2-R_{15}C* mutant did not show the slow growth phenotype on glucose, the growth defects on galactose or raffinose (42), and the inositol auxotrophy (44) characteristic for Δ*snf2* strains (Figure 6). Moreover, expression of genes (*GAL1*, *INO1* and *SUC2*) that are miss-regulated in the Δ*snf2* mutants and responsible for these growth phenotypes was not affected in the *snf2-R_{15}C* mutant (Figure 7). Similarly, expression of *SER3*, a gene repressed in *SNF2* WT strains but not in the Δ*snf2* mutant (23) was not affected in the *snf2-R_{15}C* mutant. In contrast, both the *snf2-R_{15}C* mutation and the Δ*snf2* deletion resulted in a loss of *VHT1* and *BIO5* induction (Figure 8). This characterized *snf2-R_{15}C* as a hypomorphic allele that affects Snf2p function only for Vhr1p-regulated target promoters.

Induction of Vhr1p target genes by Snf2p reveals a novel mechanism of ySWI/SNF recruitment

In summary, the protein encoded by the *snf2-R_{15}C* allele acts like WT Snf2p on most ySWI/SNF target promoters, but it is unable to induce *VHT1*, *BIO5* and putatively also other Vhr1p-regulated genes. Thus, the R_{15}C mutation and the conserved EVA domain (Figure 2 and Supplementary Figure S1) seem to be crucial for *VHT1* and *BIO5* induction, but are dispensable for the regulation of most other ySWI/SNF target genes. This suggests that the transcriptional regulator Vhr1p recruits the ySWI/SNF complex to the *VHT1* promoter via its N-terminal EVA domain.

This is in line with a report that suggested multiple functional domains for Snf2p, especially in its N-terminus (47). In fact, the N-terminal region preceding the Snf2p ATPase domain is accessible for protein/protein interactions, as a 50-amino acid stretch (also named domain 1 = residues 239–289) was shown to interact with Snf11p, another ySWI/SNF subunit (48). Moreover, the crystal structure of the bacterial Snf2p homolog RapA, the only available crystal structure of a full-length Snf2 protein, demonstrates that the N-terminus of Snf2p is freely accessible and probably points away from the DNA helix (49).

In summary, the characterization of the mutant allele *snf2-R_{15}C* reveals a novel mechanism of ySWI/SNF recruitment to its target promoters. Interactions between other subunits of the ySWI/SNF complex and transcriptional regulators have been described. The Gal4p transcription factor, for example, recruits ySWI/SNF to the *GAL1* promoter by interactions with the subunits Swi1p and Snf5p (17), and Gcn4p recruits ySWI/SNF via the subunits Snf5p, Swi3p and Snf6p (50). Interestingly, Snf2p was shown to be important for the repression of *SER3* whereas neither Swi1p nor Swi3p is required for this repression (23). For Snf2p a direct contact to or at least a close proximity with transcriptional activators has also been suggested (15); however, a specific interaction site has so far not been identified and a target gene-specific role of the ySWI/SNF ATPase unit has never been demonstrated. Obviously, the N-terminal site of Snf2p shown to be important for the induction of *VHT1* or *BIO5* is not involved in the Snf2p-dependent repression of *SER3* (Figure 8).

To our knowledge, a hypomorphic allele affecting only a subset of ySWI/SNF target genes has never been reported. The identification and characterization of the *snf2-$R_{15}C$* allele provides new insights into the mechanisms allowing gene-specific action of a machinery that is involved in the global regulation of gene expression.

SUPPLEMENTARY DATA

Supplementary Data is available at NAR Online.

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