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

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Received March 26, 2010; Revised December 31, 2010; Accepted January 1, 2011

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-R15C, 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 Δsnf2 and Δswi1 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 demonstrate 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
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 [hBra (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 NucRD (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 (R15C). Our data reveal a novel mechanism for target gene-specific ySWI/SNF recruitment in yeast via the N-terminus of Snf2p.

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**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-R15C) 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-R15C* 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-SNF2F and S2-SNF2F from plasmid pYM6 (33) and inserted via homologous recombination into the *SNF2* locus of MWY760gc yielding strain MWY760gc-SNF2-myc. The 5′-end of this modified *SNF2* sequence was amplified by PCR (ScSNF2F+5047f and TRP primer), cloned into pJet1.2, and sequenced and ligated via the BglII and XhoI restriction sites of this fragment into the BamHI/XhoI-cut yeast plasmid. The resulting plasmid was called pMW817.

A partial genomic sequence for an Snf2p-myc tag was amplified with the primers S3-SNF2F and S2-SNF2F from plasmid pYM6 (33) and inserted via homologous recombination into the *SNF2* locus of MWY760gc yielding strain MWY760gc-SNF2-myc. The 5′-end of this modified *SNF2* sequence was amplified by PCR (ScSNF2F+5047f and TRP primer), cloned into pJet1.2, and sequenced and ligated via the BglII and XhoI restriction sites of this fragment into the BamHI/XhoI-cut yeast single copy vectors pPMW814 or pPMW817 that contain WT *SNF2* or mutated *snf2-R15C* genomic sequences.

| Table 1. Yeast strains used in this study |
|-----------------------------------------|
| **Strain** | **Genotype** | **Reference/Source** |
| JS89:15-23 | MATα; his3-A1; leu2-3, trp1-289; ure2-52; can1 | (51) |
| JSYAvht1 | Isogenic to JS89:15-23; vht1::HIS3 | (26) |
| MWY760gc | Isogenic to JS91:15-23; vht1::ADH1p | (28) |
| AMYmut153 | EMS mutant of MWY760gc; snf2-153 | This study |
| BY4741 | MATa; his3-A1; leu2-3,0; met15-0; ura3-0 | EUROSCARF |
| Y01586 | Isogenic to BY4741; snf2::kanMX | EUROSCARF |
| Y01250 | Isogenic to BY4741; snf2::kanMX | EUROSCARF |
| FY3 | MATa; ura5-2 | (52) |
| YBC3010 | Isogenic to FY3; SNF2-13myc::kanMX | Bradley Cairns |
| MWY8003 | Isogenic to YBC3010; vhr1::K1URA3 | This study |
respectively. The resulting single copy plasmids with the BglII/XhoI insertions encoded Snf2p-myc or snf2p-R1/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 was used. For the 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 (pVHT1::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 Δsnf2 deletion mutant Y01586 (EUROSCARF, Frankfurt/Main, Germany) by quantitative RT-PCR (qRT-PCR; Figure 1B). In agreement with the previous data, the Δsnf2 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
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-R15C. 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-R15C 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-R15C mutation results in a reduced amount of snf2p-R15C protein, e.g. as a result of reduced stability of the mutant protein, we compared the amounts of Snf2p and snf2p-R15C proteins in yeast cells. To this end, we generated single copy plasmids encoding modified Snf2p and snf2p-R15C proteins with C-terminal myc tags (Snf2p-myc and snf2p-R15C-myc) and expressed these constructs in the Δsnf2 mutant strain Y01586. The amounts of Snf2p-myc and snf2p-R15C-myc were compared on western blots treated with anti-myc antisera (anti-myc-AB). Figure 2C shows that the amount of snf2p-R15C-myc proteins is not decreased compared with the Snf2p-myc control.
The ySWI/SNF complex is recruited to pVHT1 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 /C1 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-R15C mutation affects VHT1 expression directly, and that WT ySWI/SNF may bind directly to pVHT1.

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 Δvhr1 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 pVHT1, (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-R15C cannot occupy pVHT1

The finding that Snf2p occupies the pVHT1 (Figure 4A) suggested that this recruitment of Snf2p might be specifically affected by the mutation in the snf2p-R15C protein. To test this hypothesis, we performed additional ChIP analyses with three yeast strains expressing SNF2, SNF2-myc or snf2p-R15C-myc in the background of the Δsnf2 mutant strain Y01586 at low, VHT1-inducing biotin concentrations. Figure 4B demonstrates that as shown in Figure 4A Snf2p-myc occupies the pVHT1. Most importantly, however, Figure 4B also demonstrates that snf2p-R15C is not recruited to the VHRE. Together with the data shown in Figure 4A, this result shows that snf2p-R15C fails to induce VHT1 expression, because its
R_{15}C mutation abolishes the Vhr1p-mediated recruitment of Snf2p to the VHRE element in pVHT1.

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 \textit{snf2} mutant, of the corresponding \textit{SNF2} WT and of a \textit{vht1} deletion mutant [JSY\textit{Δvht1} (26)] on SD media with decreasing biotin concentrations. As expected, the \textit{Δvht1} mutant did grow only on high biotin (2 mg biotin l\textsuperscript{-1}; Figure 5). Also the \textit{Δsnf2} cells failed to grow on medium with low biotin (0.02 µg biotin l\textsuperscript{-1}; Figure 5). The growth difference between \textit{Δvht1} and \textit{Δsnf2} cells on medium biotin concentrations (200 µg biotin l\textsuperscript{-1}) results from a weak basal activity of pVHT1 in \textit{Δsnf2} cells and the complete absence of Vht1p activity in \textit{Δvht1} cells.

At high and medium biotin concentrations, the \textit{Δsnf2} cells grow slower than the WT (Figure 5), a well-known behavior of ySWI/SNF mutants on glucose media (42). Transformation of the \textit{Δsnf2} mutant with the pVHT1oe plasmid that expresses \textit{VHT1} under the control of the constitutive \textit{ADH1} promoter (26) complemented only the growth defect on low biotin, but not the slower growth of the \textit{Δsnf2} mutant at higher biotin concentrations (Figure 5). The same results were obtained in experiments performed with the \textit{Δswi3} deletion mutant Y01250 (not shown). In summary, these results confirmed that mutations in ySWI/SNF subunits result in a lack of biotin-dependent \textit{VHT1} induction (Figure 1B) and consequently in a reduced capacity to grow on low biotin (Figure 5).

The \textit{snf2-R_{15}C} mutant has an unusual phenotype

Compared with WT cells, \textit{Δsnf2} 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 \textit{snf2-R_{15}C} mutant did not...
containing single copy plasmid (empty plasmid YEp24). Cells were spotted on plates after serial 1:8 dilutions. snf2-R15C fails to induce SNF to the VHRE. It affects the regulation by Vhr1p and the targeting of ySWI/SNF-dependent genes (further improved by expressing WT ADH1 in all media (Figure 6). Moreover, this growth could not 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-R15C + SNF2mc) identified in the complementation screening (Figure 1A) or from the single copy plasmid (snf2-R15C + SNF2sc) used to express SNF2 in the AMYmut153 strain (Figure 1A). This demonstrated that the snf2-R15C mutation does not exhibit any of the previously published growth phenotypes of a Δsnf2 deletion mutant. It rather suggested that the snf2-R15C mutation is hypomorphic allele that specifically affects the regulation by Vhr1p and the targeting of ySWI/SNF to the VHRE.

Figure 5. Growth test on SD medium with varying biotin concentrations. The Δsnf2 mutant was transformed with plasmid pVHT1oe, expressing VHT1 from the constitutive ADH1 promoter (Δsnf2 + VHT1oe). To allow growth on the same medium, the deletion mutants Δvht1 and Δsnf2 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). Moreover, we analyzed the expression of other Vhr1p-regulated genes in WT and Δsnf2 cells. To study the expression of VHT1, GAL1 and SUC2 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 onCAA 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)].

The unexpected WT-like growth of the snf2-R15C mutant on YPGal and YPRaf medium or on SD medium w/o inositol pointed toward a normal, WT-like expression of the ySWI/SNF target genes, GAL1, SUC2 and INO1. We tested the expression of these genes by RT-PCR in the SNF2 WT, the Δsnf2 strain and the snf2-R15C 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 Δsnf2 mutants on rich medium (25). As expected, the mRNA levels of all genes were comparable in the SNF2 WT strain and in the snf2-R15C mutant (Figure 7), whereas the Δsnf2 deletion mutant failed to induce expression of GAL1, SUC2 and INO1, and did not repress SER3.

As expected, expression of VHT1 was very high in the SNF2-complemented Δsnf2 mutant, but negligible in the vector control and in the snf2-R15C-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-R15C 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-R15C and Δsnf2 differed in
their capacity to induce \textit{GAL1} on galactose medium (Figure 8C), and in their capacity to repress \textit{SER3} (Figure 8D). The expression of both genes was identical in \textit{snf2-R15C} complemented and \textit{SNF2} complemented cells. Again this confirmed that the \textit{snf2p-R15C} allele encodes a protein capable to regulate well-known ySWI/SNF target genes, such as \textit{GAL1} or \textit{SER3}. In contrast, its capacity to induce Vhr1p-regulated genes, such as \textit{VHT1} and \textit{BIO5}, is completely lost.

\section*{DISCUSSION}

The nucleosome remodeling ATPase Snf2p represents the central catalytic subunit of the ySWI/SNF complex that regulates a large number of \textit{S. cerevisiae} genes (6,7,45). In a complementation screen for transcriptional regulators of \textit{VHT1}, the gene of the biotin/H$^+$ symporter, we identified a novel \textit{snf2} mutant allele (Figure 1A). We verified the importance of Snf2p and the ySWI/SNF complex for \textit{VHT1} induction in additional analyses of \textit{\Delta snf2} and \textit{\Delta swi3} mutants (Figure 1B) and characterized \textit{VHT1} and \textit{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 \textit{VHT1} and \textit{BIO5} stay repressed under the high biotin conditions applied in these experiments.

The \textit{R15C} mutation in the newly identified \textit{snf2p} mutant allele is located in the first of two \textit{\alpha}-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 \textit{Debaryomyces hansenii} (accession number XP_461680) or \textit{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 \textit{S. cerevisiae}. 

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\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure7.png}
\caption{Comparative qRT-PCR analysis of the expression of typical ySWI/SNF target genes. RT-PCR analysis of mRNA levels of \textit{SUC2}, \textit{GAL1}, and \textit{INO1} under inducing conditions, and of \textit{SER3} under repressing conditions. The \textit{snf2-R15C} mutant strain, the \textit{\Delta snf2} deletion mutant and the WT strain were first grown in YPD medium, cultures were split and incubated in the indicated media.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure8.png}
\caption{qRT-PCR analysis of mRNA levels of \textit{VHT1}, \textit{BIO5} and \textit{GAL1} under inducing conditions and of \textit{SER3} under repressing conditions. The \textit{\Delta snf2} deletion mutant was transformed with the single copy plasmids harboring the \textit{SNF2} gene, with the \textit{snf2-R15C} mutant allele, or with the \textit{YEp24} vector (maintaining the \textit{\Delta 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 \textit{ACT1} mRNA levels; expression levels in \textit{SNF2} complemented cells were set to 100\% (\textit{n} = 3; ±SE).}
\end{figure}
Table 2. Oligonucleotides used in this study

| Oligonucleotide     | Sequence                                      |
|---------------------|------------------------------------------------|
| ScACT1g-4f          | AACAATGGATTCCTGGAATG TCCTGTGTCGGTCCTG         |
| ScACT1g+498r        | TCTTCTTGAGGATGCCCATCACC                       |
| ScACT1g+611f        | CCGTTTCTTCTCTCTCTGAAGGC                       |
| ScACT1g+813r        | AACACGCCTAAATGGAACG                          |
| ScBIOS5g+1040f      | AGGCTGCTGCTGTTTGTGTC                        |
| ScBIOS5g+1244r      | TTTCCAAGGAACCATCTT                             |
| ScGAL1c+1379        | CATATTTGCTTCTGCCTTACC                        |
| ScGAL1c+1576r       | ATAGACACGTGCCCCATGTC                         |
| ScINO1c+1316f       | CTTTACTGCTATCAGCCCTT                        |
| ScINO1c+1531r       | CTAAGGGCGTCCTTTGTTG                          |
| ScSER3c+1084f       | GAAGTGTCATGGATGTGCT                          |
| ScSER3c+1286r       | CGAGAAAATGCTTTCCAT                          |
| ScSNF2+266mut153+T  | GCAACGAGAAGGTCAATTG                          |
| ScSNF2c+5047f       | ACTTCGACCTGGTCTATGC                          |
| ScSUC2g-207r        | GATGGTTCCTGACCTCCTT                          |
| ScVHT1g-144r        | GCCTTGCATCTCATATT                                |
| ScVHT1g+607f        | GGGTCGACTTGTTGCCAT                           |
| ScVHT1g+808r        | CTGAATGCAGGAGAATCC                           |
| ScVHT1g+361f        | TGATTTGCCCTCATGTGCA                          |
| ScVHT1g+144r        | GCTTTCGATCATACTTTAT                          |
| S2-SNF2             | GTCTACGTATATACAAAAAGT                        |
| S3-SNF2             | TCTTCCACAGATAGAGGCGGC                        |
| TRP primer          | GCCATATTGAGGAGAAGGAG                         |
| YIL056Wg+1411f      | CCAACGCTGCTAGGTTG                            |
| YIL056Wg+1617r      | GGCCACTGATTATTATGAGT                         |
| YIL056W-ko-5        | TTCTAATATCCTTCTCTTGTG                      |
| YIL056W-ko-3        | AACATGTGAATGACGGATG                         |

including the less closely related Isw1p, Isw2p, Chd1p, Iono80p 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 Δvhr1 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-R15C mutant represents a hypomorphic allele

Surprisingly, the snf2-R15C 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 Δsnf2 strains (Figure 6). Moreover, expression of genes (GAL1, INO1 and SUC2) that are miss-regulated in the Δsnf2 mutants and responsible for these growth phenotypes was not affected in the snf2-R15C mutant (Figure 7). Similarly, expression of SER3, a gene repressed in SNF2 WT strains but not in the Δsnf2 mutant (23) was not affected in the snf2-R15C mutant. In contrast, both the snf2-R15C mutation and the Δdeletion resulted in a loss of VHT1 and BIO5 induction (Figure 8). This characterized snf2-R15C 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-R15C 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 R15C 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 I = residues 229–289) was shown to interact with Snfl1p, 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-R15C 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 Gen4p recruits ySWI/SNF via the subunits Snf5p, Swi3p and Snf6p (50). Interestingly, Snf2p was shown to be involved in 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).

The binding of Vhr1p to the VHT1 promoter is necessary for the induction of Snf2p expression. This is consistent with previous reports showing that VHR1 is required for the induction of GAL1 expression. The mechanism by which Vhr1p recruits the ySWI/SNF complex is still unclear. However, 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).

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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-R15C 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.

ACKNOWLEDGEMENTS
We thank Walter Weber for technical assistance and Kristin Bernhardt for experimental help. We are grateful to Brad Cairns for providing yeast strains, and to Christian Koch and Alexander Schwahn (Chair of Biochemistry, FAU Erlangen-Nürnberg) for helpful discussions.

FUNDING
This work was funded by the Deutsche Forschungsgemeinschaft (grant SA 382/17-1 to N.S.). Funding for open access charge: German Research Foundation (DFG).

Conflict of interest statement. None declared.

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