MOLECULAR AND CELLULAR BIOLOGY, Aug. 1998, p. 4707–4718 Vol. 18, No. 8
0270-7306/98/$04.00+0
Copyright © 1998, American Society for Microbiology. All Rights Reserved.

Nuclear Proteins Nut1p and Nut2p Cooperate To Negatively Regulate a Swi4p-Dependent *lacZ* Reporter Gene in *Saccharomyces cerevisiae*

RAMON K. TABTIANG AND IRA HERSKOWITZ*

Program in Biochemistry and Molecular Biology, Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94143-0448

Received 25 February 1998/Returned for modification 25 March 1998/Accepted 14 May 1998

The URS2 region of the *Saccharomyces cerevisiae HO* upstream region contains 10 binding sites for the Swi4p/Swi6p transcription factor and confers Swi4p dependence for transcription. Using a hybrid promoter, UAS_{GAL} (upstream activation sequence of *GAL1*)-URS2R, in which the *GAL1-10* regulatory region is fused to the proximal 360 bp of URS2, we isolated mutants in which Swi4p is no longer required for transcription. Mutations of *SIN4*, *ROX3*, *SRB8*, *SRB9*, *SRB10*, *SRB11*, and two novel genes, *NUT1* and *NUT2*, relieve the requirement of Swi4p for expression of this reporter. We found that *NUT1* (open reading frame [ORF] YGL151w) is a nonessential gene, that *NUT2* (ORF YPR168w) is essential, and that both Nut1p and Nut2p encode nuclear proteins. Deletion of *NUT1* causes a constitutive, Swi4p-independent phenotype only in combination with the *nut2-1* allele or an allele of *CCR4*. In contrast, inactivation of a temperature-sensitive allele of *NUT2*, *nut2-ts70*, alone causes constitutivity. *nut1*Δ *nut2-1* cells and *sin4*Δ cells exhibit Swi4p-independent expression of an *ho-lacZ* reporter but not of an intact *ho* gene. Likewise, a *pPHO5-lacZ* construct is constitutively expressed in *nut1 nut2* mutants relative to their wild-type counterparts. These results suggest that Nut1p, Nut2p, Sin4p, and Ccr4p define a group of proteins that negatively regulate transcription in a subtle manner which is revealed by artificial reporter genes.

Cells express only a subset of genes at a given time. The remaining genes are quiescent. How eukaryotic genes are maintained in a repressed state is a fundamental issue that remains poorly understood (reviewed in reference 21). Specific DNA binding proteins are known to bind promoters of some genes and inhibit gene expression. In addition, for many genes, the arrangement of nucleosomes across the promoter is thought to block transcription. Other genes are repressed due to their location in heterochromatic regions of the chromosome.

The yeast *Saccharomyces cerevisiae* is a valuable experimental organism for identifying components that maintain the quiescent state of genes. Numerous screens have been done in *S. cerevisiae* for mutations that restore or enhance transcription of inactive genes. For example, a screen for mutants that restore transcription of a *HIS4* locus that has been inactivated by a transposon insertion into its promoter has identified a group of genes known as the *SPT* (suppressor of Ty) genes (62). The *SPT* gene products include basal transcription components such as Spt15p, the TATA binding protein (17), chromatin components such as histones (11), and other proteins of undetermined function such as Spt6p, which has been shown to interact with histones (8). Nonet and Young have identified mutations which restore viability to strains whose RNA polymerase II enzyme is compromised by truncation of the carboxyl-terminal domain of the Rpb1p subunit (41). Many of the suppressors of Rpb1p truncations (*SRB*) encode proteins that copurify with the RNA polymerase II holoenzyme (20, 29, 59). For example, *SRB10* and *SRB11* encode a cyclin-dependent kinase and cyclin which copurify with the holoenzyme (26, 29).

Studies of the regulation of specific genes have also been invaluable in the search for proteins that negatively regulate transcription. For example, the *SUC2* gene is not expressed when cells are grown in glucose-containing medium. Mutations that allow expression of the *SUC2* gene in the presence of glucose have been found in the genes *SSN6*, *SRB8*, *SRB9*, *SRB10*, *SRB11*, *SIN4*, *ROX3*, and *RGR1* (54). Sin4p, Rgr1p, and Rox3p are components of a mediator activity that allows yeast RNA polymerase II to be stimulated by activators in vitro (18, 28). Biochemical studies suggest that Srb10p, Srb11p, Sin4p, Rgr1p, and Rox3p are intimately involved with RNA polymerase II function. Independently, genetic studies implicate these proteins in the negative regulation of gene expression (10, 13, 19, 46, 49, 54, 56, 61).

We have been characterizing the negative regulation of transcription of the *HO* gene in *S. cerevisiae*. *HO* is transcribed only in haploid mother cells at Start, the G_1-to-S phase transition (22, 35). Thus, *HO* is potentially repressed in haploids in daughter cells and in cells that are outside of Start. Indeed, a daughter-specific repressor of *HO*, Ash1p, has been identified (7, 53). It is not known, however, if there exist cell cycle-specific repressors of transcription (38). The 0.7-kb region, URS2 (Fig. 1, line 1), of the *HO* upstream region is required for Start-specific expression of *HO* and for dependence on the transcription factors Swi4p and Swi6p (36–38). Swi4p binds specifically to 10 sites in URS2 (3), called Swi4p cell cycle boxes (SCBs) (9, 37). Swi4p contains a specific DNA binding domain, four ankyrin repeats, and a domain for interaction with Swi6p (4, 5, 43, 51). Together Swi4p and Swi6p activate the transcription of Start-specific genes such as *CLN1*, *CLN2*, and *PCL1* as well as artificial reporters containing multimerized SCBs (3, 9, 39, 42). Both Swi4p and Swi6p are absolutely required for *HO* transcription.

* Corresponding author. Mailing address: Program in Biochemistry and Molecular Biology, Dept. of Biochemistry and Biophysics, University of California, San Francisco, 513 Parnassus Ave., San Francisco, CA 94143-0448. Phone: (415) 476-4985. Fax: (415) 502-5145. E-mail: ira@cgl.ucsf.edu.

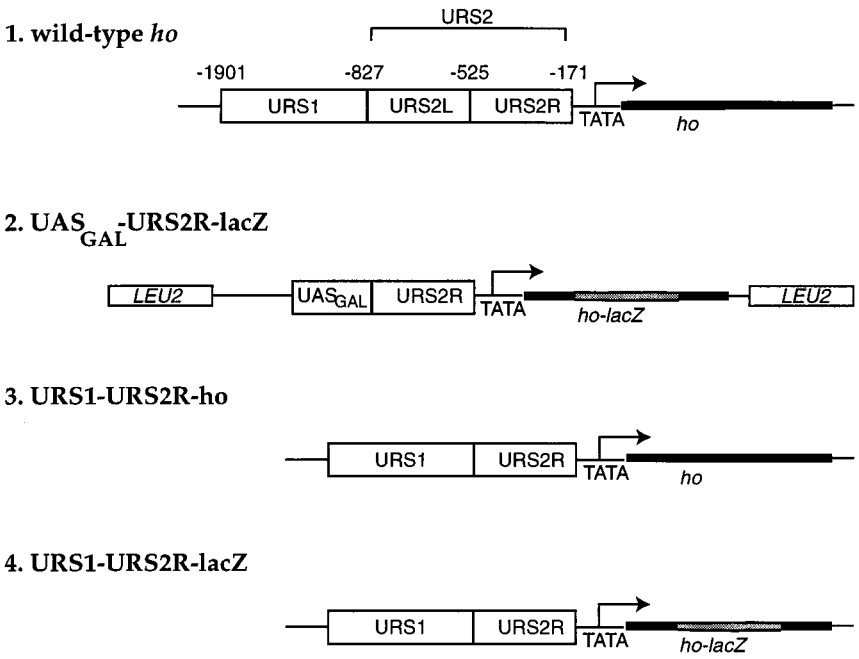

FIG. 1. Structures of the intact *HO* gene (line 1) and reporter constructs UAS_{GAL}-URS2R-*lacZ* reporter (line 2), URS1-URS2R-*ho* allele (line 3), and URS1-URS2R-*lacZ* (line 4) are depicted. Numbering indicates the nucleotide positions of the endpoints of each region relative to the *HO* ATG codon. Nucleotide positions for lines 2 to 4 are as for line 1.

The URS2 region is responsible for dependence of *HO* transcription on Swi4p and Swi6p, as deletion of URS2 allows transcription of *HO* in the absence of these proteins. Conversely, insertion of the URS2 region between the *GAL1* upstream activation sequence (UAS_{GAL}) and a TATA box confers Swi4p dependence on UAS_{GAL}-driven expression (38). Thus, in the absence of Swi4p, URS2 prevents expression of the *HO* gene despite the presence of potent activation sequences upstream, either UAS_{GAL} or URS1, the far upstream region of the *HO* promoter (Fig. 1, line 1).

To characterize regulation by URS2, we screened for mutants, named *nut* (negative regulation of URS2) mutants, which are defective in the Swi4p dependence of UAS_{GAL}-URS2R-*lacZ*, a synthetic reporter gene containing part of URS2. We describe two novel genes, *NUT1* and *NUT2*, that are required for the Swi4p dependence of UAS_{GAL}-URS2R-*lacZ*.

MATERIALS AND METHODS

Strains. Standard techniques for strain construction and mutant analysis were used (45). Key yeast strains are listed in Table 1. Except where indicated, all strains are isogenic to RT228, a strain derived from W303 and K1107 as follows. The *SWI4* locus of strain K1107 (gift of Kim Nasmyth, Institute of Molecular Pathology, Vienna, Austria) was deleted by using pJO98 (42) digested with *Eco*RI and *Sal*I to create RT211. This strain, RT211, was crossed to W303 to produce the segregant RT228. To create strains isogenic to RT228, the mating-type locus of RT228 was converted to *MAT*α by two-step gene replacement (47) using pSC9 (gift of S. Chu, University of California, San Francisco). An isogenic *SWI4* strain was created by two-step gene replacement using pRKT427.

(i) Deletion of *SIN4* and *SRB* genes. *SIN4* was deleted in a haploid strain by using plasmid M1381 (gift of David Stillman, University of Utah). The *SRB* genes were deleted in strain RT689 by using the following plasmids: to disrupt *SRB8*, pSL315 (20); to disrupt *SRB9*, pWS44-11 (gift of Marian Carlson, Columbia University) (54); to disrupt *SRB10*, pMW14 (gift of Madhu Wahi, University of California, San Francisco) (61); to disrupt *SRB11*, RY7036 (gift of Richard Young, Massachusetts Institute of Technology).

(ii) Deletion of *NUT1*. *NUT1* was deleted by using plasmid pRKT365 after digestion with *Not*I and *Sfi*I, which release the disruption cassette. Deletion of the genomic *NUT1* locus was verified by PCR.

(iii) Deletion of *NUT2*. One *NUT2* allele of RT575, an **a**/α W303 diploid, was deleted by transformation with pRKT432 after digestion with *Sal*I and *Not*I. Strains with deleted alleles were identified by PCR.

(iv) URS1-URS2R-*HO*. Plasmid pRKT619 was targeted to the *ho* locus by digestion with *Nru*I and transformation into yeast strains RT690 and RT848 followed by selection for uracil prototrophy. Loop-outs were selected on plates containing 5-fluoro-orotic acid (5-FOA) (47) and verified by PCR. Alleles generated by this procedure, which deletes URS2L, are referred to as *urs2L*Δ in Table 1 and as URS1-URS2R in Results.

Mutant isolation. Strain RT243 was mutagenized with UV irradiation to 95% inviability and plated on YEP dextrose so that all mutants were independently derived. Colonies were replica plated onto YEP galactose plates covered with Whatman no. 50 filters. After overnight growth, filters were removed and subjected to a filter β-galactosidase assay (55). Blue colonies were recovered from the original YEP dextrose plate. The 14 original mutant isolates are strains RT259 through RT271. Mutations were assigned to complementation groups by mating *mat*Δ derivatives of mutant strains to *MAT*α derivatives obtained by a standard backcross. The Nut phenotype of *mat*Δ/*MAT*α diploids was assayed by β-galactosidase filter assays on YEP galactose plates.

β-Galactosidase assays. Plate and liquid β-galactosidase assays were performed as described elsewhere (55). For assays of liquid cultures, cells were grown to mid-log phase (optical density at 600 nm of 0.1 to 1.2). Assays were performed in triplicate. Standard deviations of all reported values were less than 10% of experimental values.

Complementation and cloning of *NUT* genes. (i) *SRB* gene complementation. Plasmid pRKT439 was isolated from the Rose genomic library (44) as a plasmid that complements the mutant phenotype of RT387, a *nut7-2* mutant. To determine if strains RT259 (*nut9-1*), RT381 (*nut6-2*), and RT379 (*nut8-1*) are mutant in other *SRB* genes, these strains were transformed with plasmids containing *SRB8* (pMW18; gift of M. Wahi), *SRB9* (pWS8; gift of M. Carlson) (54), or *SRB11* (pSK5; gift of S. Kuchin and M. Carlson) (26). Complementation of the Nut^- phenotype was scored by β-galactosidase filter assays on YEP galactose plates.

(ii) *ROX3* complementation. To determine if *nut3-1* is defective in *ROX3*, *RGR1*, or *SIN4*, RT269 was transformed with *URA3* plasmids containing *ROX3* (pIT218; gift of M. Carlson) (54), *RGR1* (pM2597), and *SIN4* (pM1305; gift of D. Stillman) (24). Allelism with *ROX3* was determined by integration of *Hin*dIII-cut pIT225 (gift of M. Carlson) (54) into strain RT689. This strain was crossed to *nut3* mutant strain RT362.

(iii) *NUT1* complementation. Plasmid pRKT353 was isolated from the Rose genomic library in YCp50 (44). pRKT354, a derivative of this plasmid obtained by removing the *Sal*I-*Sph*I fragment of pRKT353, complements the Nut^- phenotype of RT271. YGL151w is the only complete reading frame on this plasmid. pRKT355, which contains a *Sal*I-*Xba*I deletion that disrupts YGL151w, lacks the

TABLE 1. Yeast strains used[a]

Strain	Genotype
W303a[b]	*MAT***a** *ade2 his3 leu2 ura3-52 trp1 can1 ho*
K1107	*HML***a** *MAT***a** *HMR***a** *his3 leu2 ura3-52 trp1 can1 ho-lacZ46 ade*$^-$
RT228	*HML***a** *MAT***a** *HMR***a** *swi4ΔHIS3 his3 leu2 ura3-52 trp1 can1 ho ade*$^-$
RT233[c]	*MAT***a** *ho::uasΔURA3::ho-lacZ*
RT241[c]	*MAT*α *SWI4 LEU2::pRKT188*
RT243	*MAT***a** *swi4ΔHIS3 NUT LEU2::pRKT188*
RT259	*MAT***a** *swi4ΔHIS3 nut9-1 LEU2::pRKT188*
RT267	*MAT***a** *swi4ΔHIS3 nut7-2 LEU2::pRKT188*
RT269	*MAT***a** *swi4ΔHIS3 nut3-1 LEU2::pRKT188*
RT271	*MAT***a** *swi4ΔHIS3 nut1-1 nut2-1 LEU2::pRKT188*
RT300[c]	*MAT***a** *swi4ΔHIS3 sin4ΔLEU2 LEU2::pRKT188*
RT335[c]	*MAT***a** *sin4ΔLEU2 ho::uasΔURA3::ho-lacZ*
RT362[c]	*MAT*α *swi4ΔTRP1 nut3-1 LEU2::pRKT188*
RT364[c]	*MAT*α *swi4ΔHIS3 nut1-1 nut2-1 LEU2::pRKT188*
RT379[c]	*MAT***a** *swi4ΔHIS3 nut8-1 LEU2::pRKT188*
RT381[c]	*MAT***a** *swi4ΔHIS3 nut6-2 LEU2::pRKT188*
RT387[c]	*MAT*α *swi4ΔTRP1 nut7-2 LEU2::pRKT188*
RT420[c]	*MAT*α *swi4ΔHIS3 nut1-1 NUT2 LEU2::pRKT188*
RT421[c]	*MAT*α *swi4ΔHIS3 NUT1 nut2-1 LEU2::pRKT188*
RT564	*MAT***a** *SWI4 NUT1 NUT2 LEU2::pRKT188*
RT575[b]	*MAT***a**/α
RT576[b]	*MAT***a**/α nut2ΔhisG::*URA*::*hisG*/*NUT2*
RT609	*MAT*α *swi4ΔHIS3 nut1ΔTRP1 nut21-609 LEU2::pRKT188*
RT688	*MAT***a** *SWI4 LEU2::pRKT229*
RT689	*MAT***a** *swi4ΔHIS3 LEU2::pRKT229*
RT692	*MAT***a** *SWI4 LEU2::pRKT208*
RT693	*MAT***a** *swi4ΔHIS3 LEU2::pRKT208*
RT718	*MAT***a** *swi4ΔHIS3 nut1ΔTRP1 nut2-1::URA3 LEU2::pRKT229*
RT720	*MAT*α *SWI4 nut1ΔTRP1 nut2-1::URA3 LEU2::pRKT229*
RT748	*MAT***a** *swi4ΔHIS3 NUT1 nut2-ts70 LEU2::pRKT229*
RT781[b]	*MAT***a** *NUT2 pRKT517*
RT783[b]	*MAT***a** *nut2ΔhisG pRKT517*
RT784	*MAT***a**/α *nut1ΔTRP1/NUT1 nut2-ts70/NUT2 LEU2::pRKT229/LEU2::pRKT229 pRKT353*
RT784-1a	*MAT*α *NUT1 nut2-ts70 LEU2::pRKT229 pRKT353*
RT784-1d	*MAT*α *nut1ΔTRP1 NUT2 LEU2::pRKT229 pRKT353*
RT784-2c	*MAT*α *nut1ΔTRP1 nut2-ts70 LEU2::pRKT229 pRKT353*
RT784-6b	*MAT*α *NUT1 NUT2 LEU2::pRKT229 pRKT353*
RT817	*MAT***a** *swi4ΔHIS3 srb10ΔURA3 LEU2::pRKT229*
RT821	*MAT***a** *swi4ΔHIS3 srb11ΔURA3 LEU2::pRKT229*
RT849[c]	*MAT***a** *srb10ΔURA3 ho::uasΔURA3::ho-lacZ*
RT850[c]	*MAT***a** *nut1ΔTRP1 nut2-1::URA3 ho::uasΔURA3::ho-lacZ*
RT854	*MAT***a** *swi4ΔHIS3 srb8ΔURA3 LEU2::pRKT229*
RT890	*MAT***a** *urs2LΔho*
RT930	*MAT***a** *swi4ΔHIS3 srb9ΔURA3 LEU2::pRKT229*
RT952	*MAT***a** *swi4ΔHIS3 NUT urs2LΔho*
RT953	*MAT***a** *swi4ΔHIS3 nut1ΔTRP1 nut2-1::URA3 urs2LΔho*
RT954[c]	*MAT***a** *swi4ΔHIS3 nut1ΔTRP1 nut2-1::URA3 urs2LΔho-lacZ*
RT973[c]	*MAT***a** *SWI4 NUT urs2LΔho-lacZ*
RT976[c]	*MAT***a** *swi4ΔHIS3 urs2LΔho-lacZ*
RT1118	*MAT***a** *swi4ΔHIS3 srb10Δ urs2LΔho*
RT1119[c]	*MAT***a** *swi4ΔHIS3 sin4ΔLEU2 urs2LΔho-lacZ*
RT1120[c]	*MAT***a** *swi4ΔHIS3 sin4ΔLEU2 urs2LΔho*

[a] All strains are isogenic to RT228 except as noted otherwise. *urs2LΔ* is referred to in the text as URS1-URS2R.
[b] Isogenic to W303.
[c] Derived from K1107 and W303.

complementing activity. To determine if the insert on pRKT353 is allelic to *NUT1*, the 2.3-kb *Hin*dIII fragment from pRKT353 was subcloned into the *Hin*dIII site of pRS306 (52) to generate pRKT356. pRKT356 was targeted to the genomic locus of the insert after digestion with *Bst*EII and transformation to uracil prototrophy in the *NUT1 NUT2* strain RT243. When the progeny from crosses of this strain to *nut1 nut2* RT364 were analyzed, only 1 of 49 *URA3* spores was phenotypically Nut$^-$, indicating that *URA3* is tightly linked to the *NUT1* locus.

(iv) *NUT2* complementation. Plasmid pRKT404 was isolated from the Rose genomic library. The *Sal*I-*Hin*dIII fragment of the genomic insert from pRKT404 was subcloned into pRS306 to generate pRKT417. Integration of *Afl*II-digested pRKT417 marks the locus of the insert with *URA3*. Analysis of 16 tetrads from a cross between *nut2-1* and a strain bearing an integration of *Afl*II-digested plasmid pRKT417 demonstrated that the insert on pRKT404 was allelic to *nut2-1* since none of 16 Nut$^-$ segregants carried the integrated pRKT417 allele. To determine which open reading frame complemented the *nut2-1* defect, derivatives of pRKT404 were made by deleting the *Eco*RI fragment (pRKT447), the *Sph*I-*Afl*II fragment (pRKT448), and the *Xba*I-*Afl*II fragment (pRKT450) and by filling in the *Afl*II site, which inactivates the reading frame YPR169w (pRKT449). This analysis demonstrated that YPR168w is the open reading frame that complemented the *nut2-1* defect.

(v) *NUT21*/*CCR4* complementation. Plasmid pRKT562 was isolated from the Rose genomic library by complementation of the mutant phenotype of RT609. The *Sal*I-*Not*I fragment was excised from pRKT562 to generate plasmid pRKT564, which still complemented the mutant phenotype. The *Spe*I-*Not*I fragment from pRKT562 was subcloned into the *Spe*I and *Not*I sites of pRS306 to

TABLE 2. Plasmids used

Name	Description
pRKT186	pBR322.*ho*::*uas*Δ*URA3*::*ho-lacZ*
pRKT208	pRS305.UAS$_{GAL}$-*lacZ*
pRKT229, pRKT188	pRS305.UAS$_{GAL}$-URS2R-*lacZ*
pRKT353	YCp50.*NUT1*
pRKT354	YCp50.*NUT1*Δ*Sal*I-*Sph*I
pRKT355	YCp50.*NUT1*Δ*Sal*I-*Xba*I
pRKT356	pRS306.2.3kb *Hin*dIII adjacent to *NUT1*
pRKT363	pGEM11.*NUT1*
pRKT365	pGEM11.*nut1*Δ*TRP1*
pRKT404	YCp50.*NUT2*
pRKT417	pRS306.*NUT2*
pRKT427	pRS306.*SWI4*
pRKT432	pBluescript.*nut2*Δ*hisG.URA3.hisG*
pRKT439	YCp50.*NUT7*/*SRB10*
pRKT447	YCp50.*NUT2*(*Eco*RIΔ)
pRKT448	YCp50.*NUT2*(*Xba*I-*Sph*IΔ)
pRKT449	YCp50.*NUT2*(*Afl*IIΔ)
pRKT450	YCp50.*NUT2*(*Xba*I-*Afl*II)
pRKT515	pRS306.*nut2-1*
pRKT517	pRS316.*NUT2*
pRKT519	YCp50.*nut2-1*
pRKT535	pRS306.*URA3.NUT2-HA*
pRKT542	pRS306.*URA3.HA-NUT1*
pRKT559	pRS306.*nut2-ts70*
pRKT562	YCp50.*NUT21*
pRKT564	YCp50.*NUT21*(Δ*Sal*I-*Not*I)
pRKT566	pRS306.*NUT21*(*Spe*I-*Not*I)
pRKT619	pRS306.*urs2L*Δ*ho*

generate plasmid pRKT566. When integrated at the *URA3* or *CCR4* locus, pRKT566 complemented the mutant phenotype. *CCR4* is the only gene contained on the *Spe*I-*Not*I fragment. Finally, this construct was used to mark the *CCR4* locus by integration of the *Sfi*I-digested plasmid. Crosses established that *CCR4* is tightly linked to *nut21-1*, since in 10 tetrads, no recombination was observed between *nut21-1* and the marked allele of *CCR4*.

Sequence analysis. Database searches with the Nut1p and Nut2p protein sequences were performed by XREF (6), with additional support from the Wisconsin package of the Genetics Computer Group. Nucleotide positions in the *HO* promoter are numbered relative to the *HO* ATG codon such that the A immediately preceding the ATG is −1.

Plasmids. Standard methods for DNA manipulations were as described previously (50). Plasmids generated for this study are listed in Table 2.

(i) *lacZ* reporters. Two UAS$_{GAL}$-URS2R-*lacZ* reporters, pRKT229 and pRKT188, were used. To create the UAS$_{GAL}$-URS2R-*lacZ* reporter pRKT229, the *Xho*I-*Hin*dIII fragment of pRKT225, which contains the 354-bp *Ssp*I-*Hin*dIII fragment of URS2 filled in and ligated into the *Sal*I site of pBluescript, was cloned into the *Xho*I and *Hin*dIII sites of pRKT208. To create pRKT188, the 406-bp *Ssp*I fragment of URS2 was ligated into the *Hin*dIII site of a plasmid closely related to pRKT208. This construct generates a small duplication of the region between the *Hin*dIII site at −171 and the *Ssp*I site at −122. pRKT208 was generated in two steps. First, the *Hin*dIII-*Bgl*II fragment of pJO11, a derivative of the *ho-lacZ* fusion from Russell et al. (48), was ligated into pRS305 (52) cut with *Hin*dIII and *Bam*HI. This plasmid was digested with *Apa*I, filled in with T4 polymerase, and ligated with the *Sau*3A-*Ava*I fragment containing UAS$_{GAL}$ which had been filled in with T4 polymerase. To create pRKT533, URS1 with an *Apa*I site and an engineered *Xho*I site (at position −871 of *HO*) was digested with these enzymes and ligated into the *Apa*I and *Xho*I sites upstream of URS2 in the reporter construct. To create pRKT619, the *Apa*I-to-*Hin*dIII fragment from pRKT533 was cloned into the *Apa*I and *Hin*dIII sites of pRS306.

(ii) UAS-less reporter. A fragment containing the *URA3* gene was ligated into a *Hin*dIII- and *Sac*I-digested pBR322 plasmid containing the *HO* upstream region and gene to generate plasmid pRKT186. pRKT186 has the *HO* upstream region replaced with *URA3*. Strain RT233 was generated by transformation of strain K1107 with *Sal*I- and *Pst*I-digested pRKT186. The resulting allele at the *HO* locus, *ho*::*uas*Δ*URA3*::*ho-lacZ*, lacks a UAS and is similar in construction to a UAS-less reporter plasmid used by others (24).

(iii) *NUT1* deletion plasmid. The 5.2-kb *Xho*I fragment containing YGL151w was subcloned into the *Sal*I site of pGEM11(f$^+$) to generate pRKT363. Plasmid CY253, containing *TRP1*, was digested with *Bgl*II and *Hin*cII and ligated to pRKT363 cut with *Bgl*II and *Eco*RV. This plasmid was then digested with *Bgl*II and *Nco*I, filled in with Klenow DNA polymerase, and religated to remove the entire 5′ coding region of YGL151w. This construct, pRKT365, deleted all of the *NUT1* open reading frame except the region coding for the carboxyl-terminal 53 amino acids.

(iv) *NUT2* deletion plasmid. The 9-kb *Sal*I-*Hin*dIII fragment was subcloned into pBluescript KS$^+$. The *Eco*RI-*Bam*HI fragment was then removed by digestion and recircularization of the vector. Divergent primers containing *Bam*HI sites that anneal at the ATG and stop codon of YPR168w were used in the PCR to amplify the flanking regions of YPR168w. The PCR product was digested with *Bam*HI and recircularized. The resulting plasmid was digested with *Bam*HI and ligated to the *Bam*HI-*Bgl*II fragment containing the *hisG-URA3-hisG* cassette from pNKY51 (1) to generate pRKT432.

(v) *NUT1* tags. The *Sac*I-*Xho*I fragment of pRKT363 containing the entire *NUT1* locus was cloned into the *Sac*I and *Xho*I sites of pRS306 (52). Uracil-substituted single-stranded DNA was prepared from this plasmid by using the phage VCSM13 (Stratagene) and *Escherichia coli* CJ236. By using site-directed mutagenesis (27), a *Bam*HI site was inserted at the amino-terminal end of the *NUT1* open reading frame. The resulting plasmid was digested with *Bam*HI, and annealed oligonucleotides encoding the hemagglutinin (HA) tag were ligated in frame. This plasmid, pRKT542, complemented a *nut1*Δ mutation.

(vi) *NUT2* tags. The *Xba*I-*Cla*I fragment containing the *NUT2* locus was subcloned into the *Xba*I and *Cla*I sites of pRS306. A *Bam*HI site at the carboxyl terminus was inserted by the same method as for *NUT1*. The HA tag was ligated into this site in frame to create plasmid pRKT535. For immunofluorescence, this construct was digested with *Bss*HII and integrated into the genomic *NUT2* locus. This plasmid, which complemented the inviability of a *nut2*Δ, is otherwise wild type in sequence.

Gap repair of *nut2-1*. The *nut2-1* allele was gap repaired from the strain RT271. Plasmid pRKT447 was digested with *Xba*I and *Bss*HII, gel purified, and transformed into RT271. The gap-repaired plasmid pRKT519 was recovered by electroporation into XL1-Blue cells (Stratagene). The sequence of the allele was determined by dideoxynucleotide sequencing using primers complementary to *NUT2*. The *Xba*I-*Cla*I fragment containing the *nut2-1* allele was subcloned into pRS306 to generate pRKT515. This plasmid was used to replace the genomic *NUT2* locus with the *nut2-1* allele, using two-step gene replacement (47).

Immunofluorescence. Indirect immunofluorescence was performed as described by Sil and Herskowitz (53). The HA-11 antibody (Babco) was used as the primary antibody at a 1:1,000 dilution.

Northern analysis. Northern analysis was performed as described by Sil and Herskowitz (53).

Temperature-sensitive alleles of *NUT2*. Temperature-sensitive alleles of *NUT2* were generated by PCR mutagenesis as described by Muhlrad et al. (34). One allele, *nut2-ts70*, was cloned into pRS306 to generate plasmid pRKT559. This plasmid was used to introduce *nut2-ts70* into the genomic *NUT2* locus by two-step gene replacement (47).

RESULTS

The UAS$_{GAL}$-URS2R-*lacZ* reporter is Swi4p dependent. To characterize the ability of URS2 to confer Swi4p dependence for transcription, we generated a reporter gene based on the UAS$_{GAL}$-URS2 allele of *HO* created by Nasmyth (38). To assay Swi4p dependence, the UAS$_{GAL}$ from the *GAL1-10* intervening region was fused to the *ho* TATA box which drives the expression of an *ho-lacZ* reporter gene. The 360 bp URS2R (right) from −528 to −171 was inserted between the UAS$_{GAL}$ and the TATA element to generate the UAS$_{GAL}$-URS2R-*lacZ* reporter (Fig. 1, line 2). An identical reporter lacking URS2R, UAS$_{GAL}$-*lacZ*, was used as a control.

Both UAS$_{GAL}$-*lacZ* and UAS$_{GAL}$-URS2R-*lacZ* were dependent on growth in galactose medium for expression (Fig. 2, lanes 1 versus 2 and 4 versus 5). However, during growth in galactose medium, UAS$_{GAL}$-URS2R-*lacZ* was not expressed in the absence of Swi4p (Fig. 2, lane 6) whereas UAS$_{GAL}$-*lacZ* was (Fig. 2, lane 3). Thus, the URS2R segment confers Swi4p-dependent expression on the UAS$_{GAL}$-URS2R-*lacZ* reporter. URS2R is close to the minimum region (−474 to −171) that confers Swi4p dependence for UAS$_{GAL}$-containing reporters (37, 58a).

Isolation of mutants that exhibit Swi4p-independent expression. To identify gene products that might function at URS2R to confer Swi4p dependence, we screened for mutations that relieve the Swi4p dependence of UAS$_{GAL}$-URS2R-*lacZ* in galactose medium. *swi4*Δ deletion strains grown on galactose plates formed white colonies because the *lacZ* reporter was not expressed, whereas mutant strains formed blue colonies due to

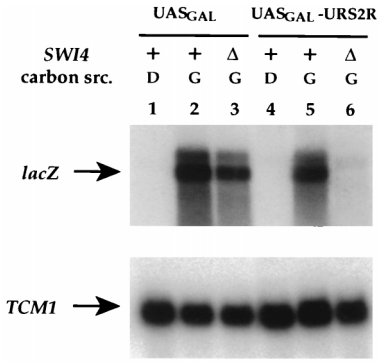

FIG. 2. The UAS$_{GAL}$-URS2R-*lacZ* reporter is dependent on Swi4p and galactose. Expression of the UAS$_{GAL}$-*lacZ* reporter (pRKT229) was assayed in a wild-type (RT692; lanes 1 and 2) and an isogenic *swi4*Δ (RT693; lane 3) strain. Expression of the UAS$_{GAL}$-URS2R-*lacZ* reporter was assayed in a wild-type (RT688; lanes 4 and 5) and an isogenic *swi4*Δ (RT689; lane 6) strain. RNA was isolated from these strains grown in YEP galactose (G; lanes 2, 3, 5, and 6) or YEP dextrose (D; lanes 1 and 4) liquid medium at 30°C. RNA was analyzed by Northern blotting and hybridized with probes for *lacZ* and *TCM1* as a control for RNA content.

lacZ expression. From 80,000 mutagenized *swi4*Δ cells, 14 mutants were isolated. Mutants were categorized as either weak suppressors or strong suppressors of the *SWI4* deletion (Table 3). For example, strain RT267, which produced 10 U of β-galactosidase activity, is representative of the weak class of suppressors. In contrast, strain RT271, which produced 80 U of β-galactosidase activity, is representative of the strong class of suppressors. The wild-type parental strain expressed less than 1 U of activity. We also noted that all mutants isolated exhibited similar secondary phenotypes. In particular, they invaded the agar of YEP dextrose plates to much greater extent than the parental strain and were more flocculent than the parental strain when grown in liquid culture.

To determine if the mutant phenotypes were due to recessive mutations, we mated *MAT* deletion derivatives of each mutant to a wild-type *MAT*α *swi4*Δ strain. All 14 mutants appeared recessive, as they did not express the UAS$_{GAL}$-URS2R-*lacZ* reporter gene. Mutations in the *lacZ* reporter are expected to be dominant. Therefore, these mutants were likely to carry mutations in genes required for transcriptional regulation of the reporter.

Each mutant was backcrossed to a *MAT*α *swi4*Δ UAS$_{GAL}$-URS2R-*lacZ* strain to determine if the defect segregated as a single-gene trait. In 9 of the 10 mutants, the defect in reporter gene regulation segregated as a single-gene trait in more than seven tetrads. However, in one strong mutant, strain RT271, the defect segregated as a two-gene trait (see below).

Because the mutations were recessive, we were able to perform complementation tests by mating *mat*Δ derivatives of each mutant with *MAT*α derivatives obtained from backcrossing. Mutations that failed to complement were provisionally assigned to the same complementation group. From this analysis (data not shown), we deduced that at least nine separate complementation groups were defined (summarized in Table 4).

Weak Nut$^-$ mutants carry mutations in the genes *SRB8*, *SRB9*, *SRB10*, and *SRB11*. To determine the nature of the defect in strains of the weak phenotype class, we cloned *NUT7* by complementation (see Materials and Methods). Sequencing of the insert of the complementing plasmid indicated that it contained *SRB10*. Mutations in this gene were previously identified as a suppressor of truncations in the carboxyl-terminal domain of RNA polymerase II, as a modifier of the glucose-repressed state of *SUC2* (54), and as a modifier of α2-mediated repression of **a**-specific genes (61). Plasmid pMW11, which contains only *SRB10* and none of the adjacent genes from the original complementing plasmid, also complemented the defect in *nut7-2*. Further, as for the original *nut7* alleles, deletion of *SRB10* had a weak defect in reporter gene regulation (Table 5).

These data strongly suggest that *nut7-2* is an allele of *SRB10* and raised the possibility that the remaining weak constitutive mutants, *nut6*, *nut8*, and *nut9*, are defective in the genes *SRB8*, *SRB9*, or *SRB11*, since mutation of these *SRB* genes causes phenotypes similar to those caused by mutations in *SRB10* (54). To test this hypothesis, we transformed *nut6*, *nut8*, and *nut9* mutants with centromere plasmids containing *SRB8*,

TABLE 3. *nut* and *sin4*Δ mutations alleviate Swi4p-dependent reporter gene expression

Genotype	β-Galactosidase activity[a]
SWI4	100
*swi4*Δ	<1
*swi4*Δ *nut7-2*	10
*swi4*Δ *nut1-1 nut2-1*	80
*swi4*Δ *sin4*Δ	270

[a] β-Galactosidase activity produced by the UAS$_{GAL}$-URS2R-*lacZ* fusion reporter was assayed in strains grown in galactose medium at 30°C and normalized such that the activity present in *SWI4*$^+$ derivatives of RT243 (19.7 Miller units) is 100. Expression of the reporter was analyzed in the *swi4*Δ parental background (RT243), in a weak (RT267) and a strong (RT271) mutant, and in a strain deleted for *SIN4* (RT300). Except for RT300, which is closely related, these strains are isogenic.

TABLE 4. Summary of genes identified

Complementation group[a]	No. of alleles	Gene	Open reading frame
NUT1	1	*NUT1*	YGL151w
NUT2	1	*NUT2*	YPR168w
NUT3	1	*ROX3*	YBL093c
NUT6	5	*SRB8*[b]	YCR081w
NUT7	2	*SRB10*[b]	YPL042c
NUT8	1	*SRB9*[b]	YDR443c
NUT9	1	*SRB11*[b]	YNL025c
NUT21	1	*CCR4*	YAL021c

[a] Mutants defective in the complementation groups *NUT4* and *NUT5* are both complemented by a high-copy-number plasmid containing *RGR1* (58a). Further analysis will be required to determine if either or both of these complementation groups are allelic to *RGR1* as mutations in *nut4* and *nut5* could exhibit intra-allelic complementation.

[b] Allelism tests were not performed.

TABLE 5. The *SRB10* class of genes is required for Swi4p-dependent reporter gene expression

Genotype	β-Galactosidase activity[a]
SWI4 SRB	100
*swi4*Δ *SRB*	0.4
*swi4*Δ *srb8*Δ	5.1
*swi4*Δ *srb9*Δ	6.6
*swi4*Δ *srb10*Δ	5.3
*swi4*Δ *srb11*Δ	4.9

[a] β-Galactosidase activity from the UAS$_{GAL}$-URS2R-*lacZ* reporter was measured in strains RT688, RT689, RT854, RT930, RT817, and RT821 during growth in YEP galactose medium at 30°C. Results were normalized so that the activity in the *SWI4 SRB* strain (55 Miller units) was 100. All strains are isogenic.

TABLE 6. Mutations in both *NUT1* and *NUT2* are required for Swi4p-independent reporter expression

Genotype	β-Galactosidase activity[a]
SWI4 NUT1 NUT2	100
*swi4*Δ *NUT1 NUT2*	1.4
*swi4*Δ *nut1-1 NUT2*	2.6
*swi4*Δ *NUT1 nut2-1*	0.8
*swi4*Δ *nut1-1 nut2-1*	108

[a] β-Galactosidase activity from the UAS_{GAL}-URS2R-*lacZ* reporter was measured in strains RT241, RT243, RT420, RT421, and RT271 during growth in YEP galactose medium at 30°C. Results are normalized so that the activity in the *SWI4 NUT1 NUT2* strain (20.6 Miller units) is 100.

SRB9, and *SRB11*. *SRB8* complemented the Nut$^-$ phenotype of *nut6-2*; *SRB9* complemented *nut8-1*; *SRB11* complemented *nut9-1*. Furthermore, deletion of *SRB8*, *SRB9*, or *SRB11* caused a partial relief of the Swi4p dependence of the UAS_{GAL}-URS2R-*lacZ* reporter (Table 5). These results show that the genes *SRB8*, *SRB9*, *SRB10*, and *SRB11* are required for appropriate regulation of the reporter.

Mutation of *ROX3* or *SIN4* causes a Nut$^-$ phenotype. To determine the identities of the remaining genes, we compared the phenotype of the *nut* mutants with that of a *sin4*Δ deletion in our strain background. Loss of *SIN4* function has been previously shown to bypass the Swi4p requirement for transcription of *ho-lacZ* reporters (24, 32). Likewise, deletion of *SIN4* allowed transcription of the UAS_{GAL}-URS2R-*lacZ* reporter in *swi4*Δ strains (Table 3). The *nut3* mutant complemented a *sin4*Δ strain, suggesting that *nut3* is not an allele of *SIN4*. Since mutations in *RGR1* and *ROX3* have phenotypes like mutations in *SIN4* (13, 23, 54), we tested whether centromere plasmids bearing these genes could complement *nut3-1*. A plasmid that contained the *ROX3* open reading frame alone complemented the mutant phenotype, whereas plasmids containing *RGR1* or *SIN4* did not. Further, we found that *nut3* was allelic to the *ROX3* gene since *nut3* segregated away from a *ROX3* locus that was marked with the *URA3* gene in eight tetrads. Therefore, mutation of either *ROX3* or *SIN4* causes inappropriate expression of the UAS_{GAL}-URS2R-*lacZ* reporter.

Mutant RT271 is defective in two genes, *NUT1* and *NUT2*. The mutant phenotype in RT271, which exhibits a strong defect in the Swi4p dependence of UAS_{GAL}-URS2R-*lacZ*, segregated as if it were due to mutations in two unlinked loci that we designated *nut1* and *nut2*. In 32 tetrads, 7 parental ditype tetrads, 4 nonparental ditype tetrads, and 21 tetratype tetrads were observed. This ratio is consistent with the expected 1:1:4 ratio for segregation of two unlinked genes, when at least one gene is far from its centromere. Further, when one mutant locus, *NUT1*, was homozygous whereas the other locus, *NUT2*, was heterozygous, single-gene segregation (2:2 segregation) of the phenotype was observed in 14 tetrads.

These data indicated that two unlinked loci must be mutated to allow expression of the reporter construct in the absence of Swi4p. By quantitating β-galactosidase activity, we determined that expression was at background levels when only one of the two loci was mutated in *nut1 NUT2* or *NUT1 nut2* strains (Table 6). In contrast, expression was robust, to the levels of strains with Swi4p activity, in a strain in which both loci were mutated. Therefore, the genes *NUT1* and *NUT2* both contribute to the negative regulation of UAS_{GAL}-URS2R-*lacZ* reporter in strains deleted for *SWI4*.

***NUT1* encodes a novel nonessential protein.** To clone *NUT1* and *NUT2*, we transformed the double mutant strain with a genomic library, reasoning that a centromere plasmid containing either gene should restore the Swi4p dependence of UAS_{GAL}-URS2R-*lacZ*. We obtained two plasmids that differed in their restriction maps. The insert from one plasmid was tightly linked to *nut1*, whereas the insert of the other plasmid was tightly linked to *nut2* (see Materials and Methods).

Subcloning of the *NUT1* insert delimited the complementing activity to a single open reading frame, YGL151w, which encodes a large polypeptide of 1,132 amino acids and has no significant homologs. Deletion of *NUT1* caused the same phenotype as the original *nut1-1* allele. Specifically, in the absence of *SWI4*, deletion of *NUT1* alone did not cause reporter gene expression in strains wild-type for *NUT2* but did permit high-level expression of the reporter in strains that carried the *nut2-1* mutation (data not shown). In conclusion, *NUT1* encodes a large novel protein which is not essential for cell viability.

***NUT2* encodes a novel essential protein.** By subcloning the insert from the other complementing plasmid, we determined that the *NUT2* gene is YPR168w. This open reading frame encodes a protein of 157 amino acids which has sequence homologs of unknown function including the human expressed sequence tag yx99c06.r1 and the *Caenorhabditis elegans* open reading frame T09A5.6 (Fig. 3).

To determine the phenotype of complete loss of function of *NUT2*, we generated a marked deletion of *NUT2* in a diploid yeast strain. Upon sporulation, only two spores of each tetrad formed colonies of 10 tetrads analyzed. These spores were invariably *NUT2* since they did not bear the marker from the deletion. Therefore, the spores that failed to form colonies lacked the *NUT2* gene, although these spores did germinate, as verified by microscopic inspection. A further indication that *NUT2* is essential is the inability of *nut2*Δ strains to lose a *URA3* centromere plasmid containing the *NUT2* gene. These *nut2*Δ strains were unable to grow in the presence of 5-FOA, which selects against the *URA3 NUT2* plasmid, although their isogenic *NUT2* siblings readily lost this plasmid (Fig. 4A). Thus, the *NUT2* gene is essential for viability.

Given that we were unable to analyze the phenotype of the *NUT2* deletion with respect to reporter gene transcription, we confirmed that YPR168w is the *NUT2* locus by rescuing the *nut2-1* allele from yeast by gap repair (see Materials and Methods). When the *NUT2* locus in a *swi4*Δ *nut1*Δ strain was replaced with the recovered *nut2-1* allele, we found that the UAS_{GAL}-URS2R-*lacZ* reporter was expressed despite the absence of Swi4p. Sequencing of the recovered allele revealed that *nut2-1* has a single nucleotide change in YPR168w that converts codon 132 from GAA to AAA, which changes a glutamic acid residue to lysine (Fig. 3). No other mutations were found. This observation confirms that YPR168w is the open reading frame mutated in *nut2-1*.

Nut1p and Nut2p localize to the cell nucleus. To determine the subcellular localization of Nut1p and Nut2p, we introduced epitope tags into the coding sequence of each gene. An amino-terminal fusion of the Nut1p open reading frame joined to two copies of the HA epitope complemented the Nut$^-$ phenotype of *nut1*Δ *nut2-1* strains. Likewise, the Nut2p open reading frame was fused in frame at its carboxyl terminus to two copies of the HA epitope. The Nut2p-HA protein complemented the inviability of a *NUT2* deletion. By indirect immunofluorescence using a monoclonal antibody directed against the HA epitope, both the HA-Nut1p fusion protein (Fig. 5, columns 1 and 2) and the Nut2p-HA protein (Fig. 5, columns 3 and 4) were localized to the nuclei of yeast cells. Little if any background signal was observed in untagged strains analyzed in

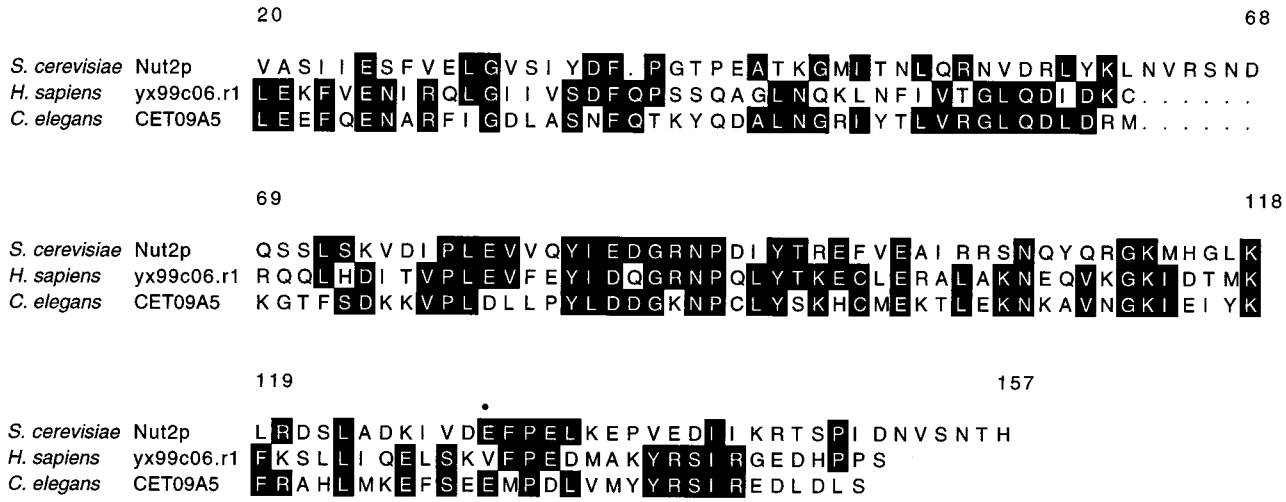

FIG. 3. Alignment of Nut2p with human and *C. elegans* homologs. The human expressed sequence tag yx99c06.r1 (GenBank accession no. N40234) and the *C. elegans* open reading frame T09A5.6 (SwissProt P45966) are aligned with Nut2p. Black highlighting indicates positions conserved among these proteins. The amino acid positions of the Nut2p sequence are indicated above the alignment. ● denotes the position mutated to lysine in the *nut2-1* allele.

parallel with the same antibodies and under the same conditions (Fig. 5, columns 5 and 6). These data suggest that Nut1p and Nut2p are predominantly localized in cell nuclei, where they might affect gene expression.

Loss of function of *NUT2* alone causes Swi4p-independent reporter transcription. To determine the phenotype of complete loss of *NUT2* function, we generated alleles of *NUT2* that are temperature sensitive for viability using PCR mutagenesis. One such allele, *nut2-ts70*, did not support cell growth at the restrictive temperature of 37°C (Fig. 4B, top right sector). *nut2-ts70* cells died with abnormal morphology but no specific cell cycle arrest (58a). We generated *swi4*Δ strains carrying the UAS_{GAL}-URS2R-*lacZ* reporter that were *NUT1 NUT2*, *nut1*Δ *nut2-1*, or *NUT1 nut2-ts70*. Following growth in YEP galactose medium at 25 or 37°C, we assayed reporter gene expression by Northern analysis (Fig. 6A). Consistent with data from analysis of β-galactosidase activity, the *lacZ* transcript was apparent in *SWI4* strains but absent from *swi4*Δ strains regardless of temperature (Fig. 6A; compare lanes 1 and 2 with lanes 3 and 4). Deletion of *NUT1* in combination with the *nut2-1* allele re-

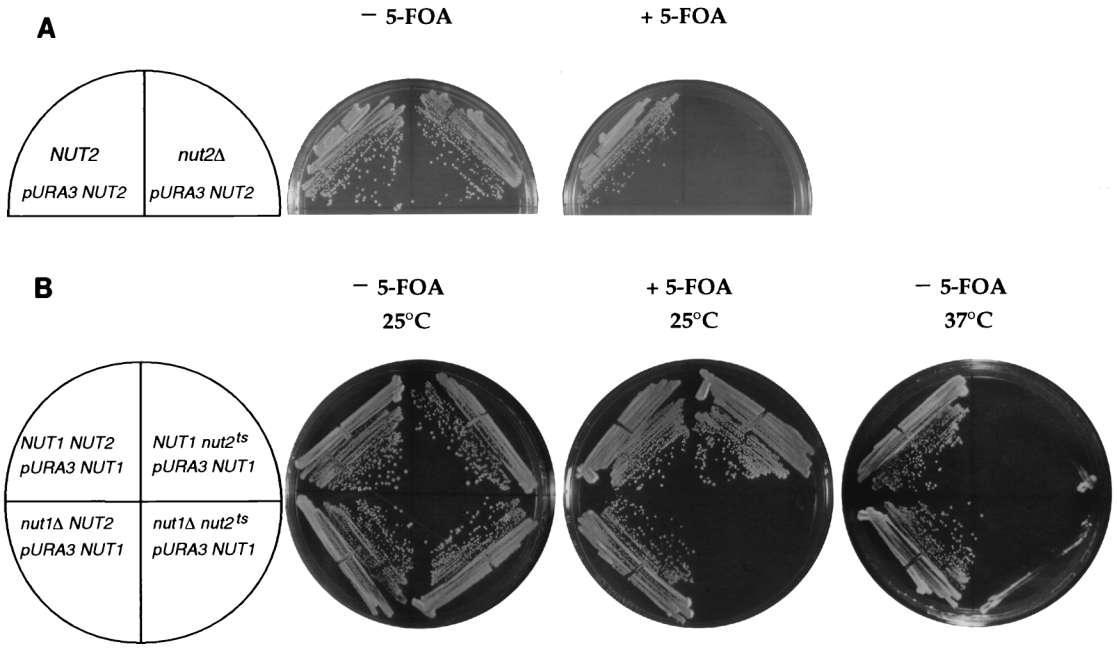

FIG. 4. *NUT2* is an essential gene that exhibits synthetic lethality with *NUT1*. (A) *NUT2* is an essential gene. Isogenic strains that are *NUT2* (RT781) or *nut2*Δ (RT783) were maintained with a centromere plasmid containing *URA3* and *NUT2*. Strains were streaked on synthetic complete medium (left) or on synthetic complete medium containing 5-FOA (right) and incubated for 3 days. (B) A temperature-sensitive allele of *NUT2*, *nut2-ts70*, is synthetically lethal with a deletion of *NUT1*. Isogenic strains derived from RT784 that are *NUT1 NUT2* (RT784-6b), *nut1*Δ *NUT2* (RT784-1d), *NUT1 nut2-ts70* (RT784-1a), and *nut1*Δ *nut2-ts70* (RT784-2c) were constructed bearing pRKT353, a centromere plasmid containing the *URA3* and *NUT1* genes. Strains were streaked on plates with (center) or without (left and right) 5-FOA and incubated at 25°C (left and center) or 37°C (right) for 3 days.

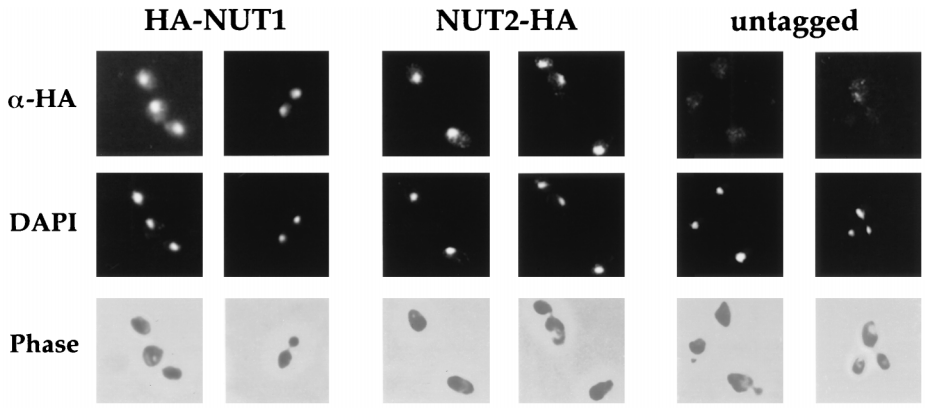

FIG. 5. Nut1p and Nut2p are localized to nuclei. Cells bearing integrated copies of the *HA-NUT1* fusion (pRKT542; columns 1 and 2), the *NUT2-HA* fusion (pRKT535; columns 3 and 4), or no fusion (columns 5 and 6) were harvested in mid-log phase, fixed, and stained with antibodies against the HA epitope (α-HA) as described in Materials and Methods. Localization of the tagged epitope was revealed by rhodamine-conjugated anti-mouse antibodies in the first row. For the same field of cells, DNA was visualized by 4′,6-diamidino-2-phenylindole (DAPI) staining in the second row. Cell outlines were visualized by phase-contrast microscopy in the third row.

stored transcription of the reporter in the absence of Swi4p (Fig. 6A, lanes 5 and 6). The *nut2-ts70* allele did not perturb reporter gene expression at the permissive temperature (Fig. 6A, lane 7) but did cause expression of reporter gene transcription at the restrictive temperature of 37°C (Fig. 6A, lane 8), despite *NUT1* activity. We conclude that inactivation of *NUT2* alone is sufficient to cause Swi4p-independent expression of the UAS_{GAL}-URS2R-*lacZ* reporter.

***NUT1* is synthetically lethal with a temperature-sensitive allele of *NUT2*.** After replacement of *nut2-ts70* into the genomic *NUT2* locus, we sporulated a *nut1*Δ/+ *nut2-ts70*/+ diploid to determine the phenotype of *nut1*Δ *nut2-ts70* double mutants. Of 19 tetrads germinated at the permissive temperature, 25°C, no *nut1*Δ *nut2-ts70* double mutants were found although 19 *NUT1 nut2-ts70* spores grew up normally. To determine if *nut1*Δ is indeed synthetically lethal with *nut2-ts70*, we obtained a double mutant that was rescued by a *URA3 NUT1* plasmid. Such double mutants were unable to lose the *URA3 NUT1* plasmids, as they failed to grow on 5-FOA whereas their isogenic *NUT1 nut2-ts70* siblings did (Fig. 4B).

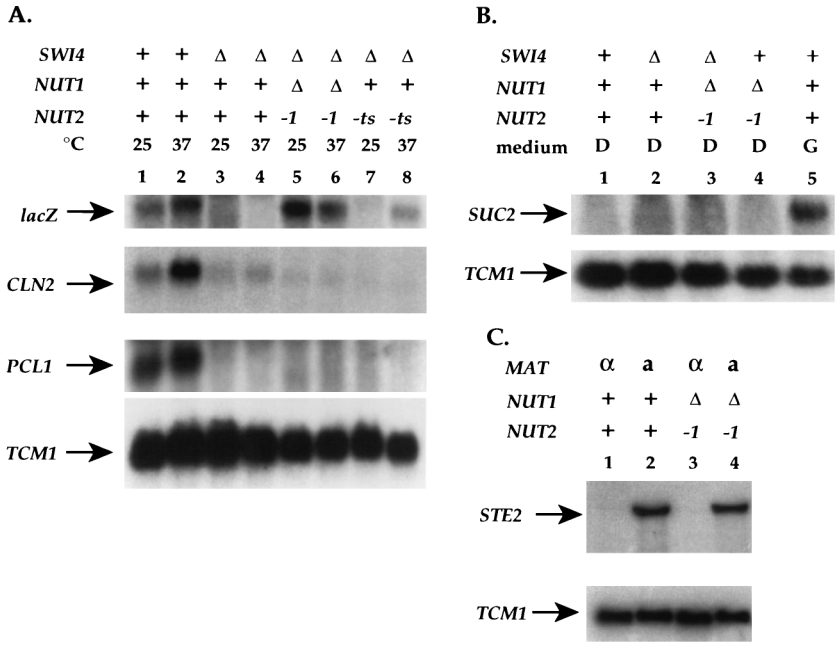

FIG. 6. Inactivation of a temperature-sensitive allele of *NUT2* allows Swi4p-independent expression. (A) Expression of the UAS_{GAL}-URS2R-*lacZ* reporter was assayed in wild-type (RT688; lanes 1 and 2), *swi4*Δ (RT689; lanes 3 and 4), *swi4*Δ *nut1*Δ *nut2-1* (RT718; lanes 5 and 6), and *swi4*Δ *NUT1 nut2-ts70* (RT748; lanes 7 and 8) strains. RNA was isolated from these strains after growth in YEP galactose liquid medium at 25°C (odd-numbered lanes) or 37°C (even-numbered lanes) for 3 h. RNA was analyzed by Northern blotting and hybridized with probes for *lacZ*, *CLN2*, *PCL1*, and *TCM1*. The latter serves as an RNA loading control. The *TCM1* signal indicates that lanes 2, 3, and 4 contain slightly more RNA than other lanes. All strains are isogenic. (B) Expression of the *SUC2* gene was assayed in wild-type (RT688; lanes 1 and 5), *swi4*Δ (RT689; lane 2), *swi4*Δ *nut1*Δ *nut2-1* (RT718; lane 3), and *SWI4 nut1*Δ *nut2-1* (RT720; lane 4) strains by Northern analysis. Strains were grown in dextrose (D; lanes 1 to 4) or in galactose (G; lane 5) medium. (C) *STE2* expression was assayed in *MAT*α cells (lanes 1 and 3) and *MAT***a** cells (lanes 2 and 4) that were either wild type (lanes 1 and 2) or *nut1*Δ *nut2-1* mutants (lanes 3 and 4).

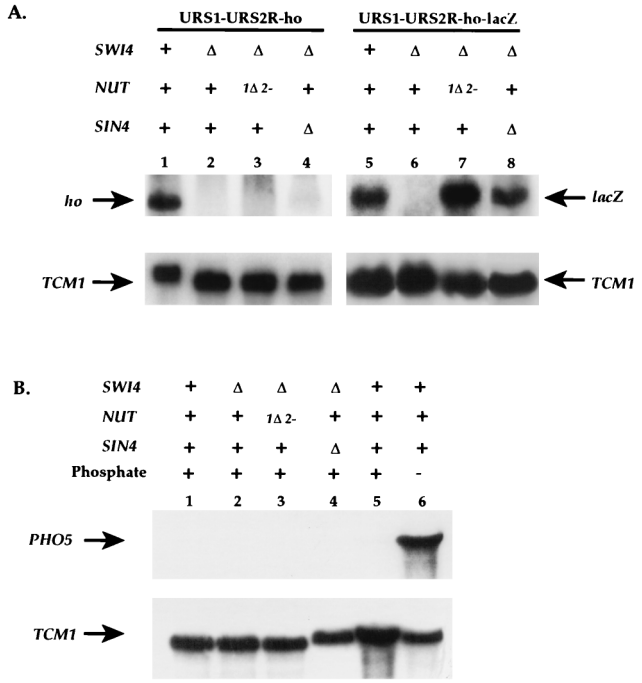

FIG. 7. The phenotype of *nut1 nut2* mutants is manifest only in the presence of *lacZ* sequences. (A) *HO* expression was analyzed for strains containing the URS1-URS2R-*ho* allele (lanes 1 to 4) or the URS1-URS2R-*lacZ* allele (lanes 5 to 8). Wild-type strains (RT890 [lane 1] and RT973 [lane 5]) were compared with *swi4*Δ strains (RT952 [lane 2] and RT976 [lane 6]), *swi4*Δ *nut1*Δ *nut2-1* strains (RT953 [lane 3] and RT953 [lane 7]), and *swi4*Δ *sin4*Δ strains (RT1118 [lane 4] and RT1120 [lane 8]). (B) *PHO5* expression was measured in strains grown in the presence (lanes 1 to 5) or absence (lane 6) of inorganic phosphate. Wild-type (lanes 1, 5, and 6) was compared with *swi4*Δ (RT952; lane 2), *swi4*Δ *nut1*Δ *nut2-1* (RT953; lane 3), and *swi4*Δ *sin4*Δ (RT1118; lane 4) strains.

Thus, deletion of *NUT1* exacerbates the growth phenotype of the *nut2-ts70* allele just as it exacerbates the reporter gene phenotype due to the *nut2-1* allele.

Mutation of *NUT1* and *NUT2* affects expression of the UAS$_{GAL}$-URS2R-*lacZ* reporter but not the endogenous *HO* gene. To determine if *NUT1* and *NUT2* are physiological regulators of *HO* transcription, we assayed *HO* transcription in the *nut1*Δ *nut2-1* double mutant. We observed that the *nut1*Δ *nut2-1* double mutant did not affect the Swi4p dependence of the intact *ho* gene containing URS1-URS2-*ho* (data not shown). Because the *lacZ* reporter only contained part of URS2, we constructed an allele of the endogenous *ho* gene in which the wild-type URS2 region was truncated, leaving only URS2R, the minimal region of URS2 required for Swi4p dependence (Fig. 1, line 3). This allele, URS1-URS2R-*ho*, required Swi4p (Fig. 7, lane 2) and Swi5p (data not shown) for *ho* transcription as assayed by Northern hybridization. However, in contrast to the UAS$_{GAL}$-URS2R-*lacZ* reporter, transcription of *ho* from the URS1-URS2R-*ho* gene was not restored in the absence of Swi4p in the *nut1*Δ *nut2-1* mutant (Fig. 7, lane 3).

To analyze the differential effects of *NUT1* and *NUT2* on URS1-URS2R-*ho* (Fig. 1, line 3) and UAS$_{GAL}$-URS2R-*lacZ* (Fig. 1, line 2), we constructed another *ho* allele that was identical to URS1-URS2R-*ho* in all respects except that the *HO* open reading frame was fused to the *E. coli lacZ* gene within the coding region of the gene (Fig. 1, line 4). We observed that expression of this construct, URS1-URS2R-*lacZ*, was also dependent on Swi4p (Fig. 7, lane 6) and Swi5p for activity. Unlike URS1-URS2R-*ho*, URS1-URS2R-*lacZ* was transcribed in the absence of Swi4p activity when the strain was *nut1*Δ *nut2-1* (Fig. 7, lane 7). Thus, the difference between URS1-URS2R-*ho* and the reporter UAS$_{GAL}$-URS2R-*lacZ* can be attributed to the presence of *lacZ* sequences. This phenotype is similar to the phenotype of *SIN4* mutants, which also bypass the requirement of Swi4p for *HO* expression only when the *HO* open reading frame is fused to *lacZ* sequences (Fig. 7, lane 4 versus lane 8) (24, 33, 40). In summary, Nut1p and Nut2p appear essential for the Swi4p dependence of *HO* alleles compromised by the presence of *lacZ* sequences.

Mutation of *NUT1* and *NUT2* affects the expression of another *lacZ*-containing reporter but not other Swi4p-dependent genes. To determine if the *nut1*Δ *nut2-1* strain is generally defective in the regulation of gene expression or defective in the regulation of *lacZ*-containing reporters other than UAS$_{GAL}$-URS2R-*lacZ*, we assayed the expression of another reporter and other genes in this strain. Transcription of the *PCL1* gene is dependent on Swi4p (42), whereas transcription of the *CLN2* gene is largely but not completely dependent on Swi4p (14). To determine if mutation of *NUT1* or *NUT2* enhances the Swi4p-independent transcription of *PCL1* or *CLN2*, we analyzed transcription of these genes in wild-type, *swi4*Δ, *swi4*Δ *nut1*Δ *nut2-1*, and *swi4*Δ *nut2-ts70* strains (Fig. 6A). We were unable to detect any increase in the Swi4p-independent transcription of these two genes in the *nut* mutant strains.

We also examined the regulation of genes whose expression is independent of Swi4p but whose repression is dependent on *SIN4* or *SRB10*. The glucose repression of *SUC2* transcription is perturbed by mutation of *SIN4* or *SRB10* (54). We found, however, that *SUC2* expression was appropriately repressed in a *nut1*Δ *nut2-1* mutant when cells were grown in glucose (Fig. 6B). The repression of *STE2* by α2 is perturbed by mutations in *SIN4* and *SRB10* (61). The *nut1*Δ *nut2-1* double mutant did not perturb *STE2* repression in α cells (Fig. 6C). Finally, the repression of *SPO13* in haploid vegetative cells was also unaffected by mutations in *NUT1* and *NUT2* (data not shown).

Although mutation of *NUT1* and *NUT2* did not affect the expression of the three genes assayed here, we found that *nut1*Δ *nut2-1* double mutants, like *sin4*Δ mutants, exhibit a greater than 10-fold elevated expression of a *pPHO5-lacZ* reporter construct (Table 7, column 2). Under repressing conditions for *PHO5* regulation, deletion of *SIN4* is known to cause increased expression of this *pPHO5-lacZ* reporter while not perturbing repression of a *PHO5* gene lacking the *lacZ* moiety (21b). Likewise, the *nut1*Δ *nut2-1* double mutant did not perturb repression of the endogenous *PHO5* gene (Fig. 7B). These data demonstrate that mutation of *NUT1* and *NUT2* affects *pPHO5-lacZ* similarly to mutation of *SIN4*. Finally, mutation of *SIN4* increases expression of reporters lacking a UAS (Table 7, column 3). Again, *nut1*Δ *nut2-1* double mutants have

TABLE 7. Mutation of *SIN4* or of *NUT1* and *NUT2* elevates expression of *pPHO5-lacZ*

Genotype	β-Galactosidase activity[a]	
	pPHO5-lacZ[b]	UAS-less *lacZ*[c]
NUT$^+$	1.0	0.1
*srb10*Δ	2.8	0.1
*sin4*Δ	11	5.2
*nut1*Δ *nut2-1*	16	1.1

[a] Normalized so that the activity in the *swi4*Δ strain (1.6 Miller units) is 1.0.
[b] Measured in strains RT952, RT1118, RT1120, and RT953 during growth under selection at 30°C in standard synthetic medium which is repressing for *pPHO5-lacZ*.
[c] Measured in strains RT233, RT849, RT335, and RT850.

TABLE 8. Mutation of *NUT21* synergizes with *nut1*Δ to cause Swi4p-independent reporter gene expression

Genotype	β-Galactosidase activity[a]
SWI4 NUT1 NUT21	100
*swi4*Δ *NUT1 NUT21*	0.4
*swi4*Δ *nut1*Δ *NUT21*	5.2
*swi4*Δ *nut1*Δ *nut21-1*	46
*swi4*Δ *NUT1 nut21-1*	0.3

[a] β-Galactosidase activity from the UAS_{GAL}-URS2R-*lacZ* reporter was measured in strains RT858, RT243, RT569, RT789, and RT709 during growth in YEP galactose medium at room temperature. Results are normalized so the activity in the *SWI4 NUT1 NUT2* strain (13.2 Miller units) is 100.

similar phenotypes, although they exhibit only a 12-fold increase, compared to a 60-fold increase caused by mutation in *SIN4*.

Mutation of *CCR4* in combination with deletion of *NUT1* has a Nut⁻ phenotype. Because *NUT2* is essential for cell viability and is a novel gene, we aimed to identify other genes that function like *NUT2*. Therefore, we sought other mutants with a Nut2p-like phenotype by screening for constitutive expression of the UAS_{GAL}-URS2R-*lacZ* reporter in cells deleted for *SWI4* and *NUT1*. From this screen, we identified genes that define mutations in at least four different complementation groups (21a). One mutant strain, RT609, was characterized further. This strain contained mutation, designated *nut21-1*, which exhibits a temperature-sensitive growth defect that cosegregated in seven tetrads with the defect in reporter gene regulation. *nut21-1* was not allelic to *NUT2* but, like the *nut2-1* allele, exhibited a Nut⁻ phenotype in combination with *nut1*Δ (Table 8). Further, *nut21-1* exhibited no phenotype alone or in combination with *nut2-1*. These results suggest that *NUT21* encodes an essential protein that, like Nut2p, functions with Nut1p to regulate expression of the UAS_{GAL}-URS2R-*lacZ* reporter.

From a centromere genomic library, we isolated a plasmid, pRKT562, that complemented both the defect in reporter gene regulation and the temperature sensitivity of the *nut21-1* mutation. Subcloning of the insert on this plasmid established that the gene *CCR4* was responsible for the complementing activity (see Materials and Methods). Furthermore, *CCR4* was linked to the *nut21-1* mutation, as no recombinants between the two loci were obtained in 10 tetrads. These data suggest that mutation of *CCR4* in combination with loss of *NUT1* function causes Swi4p-independent expression of the UAS_{GAL}-URS2R-*lacZ* reporter. Thus, like Nut2p, Ccr4p might function with Nut1p to negatively regulate UAS_{GAL}-URS2R-*lacZ*.

DISCUSSION

We have identified a novel class of genes which includes *NUT1*, *NUT2*, and *CCR4* in screening for mutations that relieve the Swi4p dependence of an artificial reporter regulated by the URS2R region of the *HO* promoter. Nut1p, Nut2p, and Ccr4p formally behave as negative regulators of gene expression. Their function is distinctive in two respects. First, these proteins contribute substantially to the regulation of artificial reporters but not detectably to their endogenous counterparts. Second, these proteins function cooperatively with each other.

Nut1p and Nut2p behave as a negative regulators of transcription. We found that the 360-bp URS2R segment from the URS2 region is sufficient to confer Swi4p dependence on the UAS_{GAL} activation sequences. We propose that in the absence of Swi4p, regulatory mechanisms inhibit activation of transcription by Gal4p in the case of UAS_{GAL}-URS2R-*lacZ* and by Swi5p in the case of *HO*. In *nut1 nut2* double mutants, however, URS2R is unable to inhibit transcription of UAS_{GAL}-URS2R-*lacZ* and URS1-URS2R-*lacZ* in the absence of Swi4p. Thus, for these reporters, Nut1p and Nut2p act as negative regulators of transcription.

This interpretation is also consistent with two additional findings. First, mutations in *SRB8*, *SRB9*, *SRB10*, *SRB11*, *SIN4*, *ROX3*, and *RGR1* also cause loss of Swi4p-dependent regulation of UAS_{GAL}-URS2R-*lacZ* (Table 4). Given that these genes are required for the repression of a number of endogenous yeast genes including *SPO13* (58), *IME1* (13), *SUC2* (54), and *STE2* (61), Nut1p and Nut2p might be similarly required for repression of transcription even though Nut1p and Nut2p are not required for the repression of *SPO13*, *SUC2*, and *STE2*. Second, both Nut1p and Nut2p are localized to the nucleus, where they would be available to regulate transcription directly.

The phenotypes of *nut1*Δ *nut2-1* mutants are context dependent. We found that the *nut1 nut2* double mutation relieved the Swi4p dependence of UAS_{GAL}-URS2R-*lacZ* and URS1-URS2R-*lacZ* but did not affect the Swi4p dependence of URS1-URS2R-*ho* or URS1-URS2-*ho*. These data establish that Nut1p and Nut2p are not absolutely required to regulate transcription of the endogenous *HO* gene. Possibly, unknown components operate redundantly with Nut1p and Nut2p to regulate *HO* transcription. The insertion of *lacZ* sequences might disable these components and thus reveal a requirement for Nut1p and Nut2p. Another possibility is that the presence of *lacZ* sequences in yeast may simply generate a new situation which requires additional proteins for regulation. We cannot exclude the possibility that mutations in *NUT1* and *NUT2* would increase the expression of any *lacZ*-containing reporter. Further experimentation will be necessary to resolve this issue.

In any event, the finding that the *nut1 nut2* double mutation primarily affects *lacZ*-containing reporters suggests that *NUT1* and *NUT2* may be members of a small group of genes whose phenotypes are limited to artificial reporters. Mutations in *SIN4* allow Swi5p-independent expression of a URS1-URS2-*lacZ* allele but not of URS1-URS2-*ho* (24, 33, 40). Mutations in *SIN4* also cause elevated expression of a phosphate-repressed *pPHO5-lacZ* construct but not of an identical construct in which the *PHO5* promoter directs transcription of the *PHO5* gene (21b). We observed that the *NUT1* and *NUT2* genes are similarly required for the repression of *pPHO5-lacZ* but do not affect the repression of the endogenous *PHO5* gene. Harashima et al. have observed that *SIN4* mutations allow constitutive transcription of the *PHO5* gene when the *PHO5* promoter and open reading frame are integrated at the *URA3* locus (19).

Thus, the requirement for Nut1p, Nut2p, and Sin4p is revealed only when the chromosomal environment for gene expression is perturbed by the insertion of bacterial *lacZ* sequences or by the removal of the gene to another genomic location. One explanation is that these alterations disrupt the normal organization of chromatin across promoter regions. Nucleosomes are important for the regulation of both *HO* and *PHO5* transcription (2, 25, 57). Conceivably, the organization of nucleosomes across the promoters of these genes requires either a suitable chromosomal environment or Nut1p and Nut2p activity. When the chromosomal milieu is disrupted, Sin4p, Nut1p, and Nut2p activity would be essential for positioning the nucleosomes that inhibit transcription at these promoters. Because other models also adequately explain the data, it remains to be determined why the phenotypes of mutations in *NUT1*, *NUT2*, and *SIN4* are evident only in artificial situations.

Nut1p and Nut2p function cooperatively. The other unusual property of the *nut1 nut2* double mutation is the strong synergy between loss of *NUT1* function and the *nut2-1* allele (Table 6). An analogous relationship was observed for genes required for glucose repression of *SUC2* (60). The finding of two-gene traits often implies functional redundancy between the products of the two genes. Because the original *nut1-1* and *nut2-1* alleles exhibit no phenotype alone, Nut1p and Nut2p may function in redundant, parallel pathways.

Instead of a simple parallel relationship, we propose that Nut2p is the primary functional moiety whereas Nut1p is a dispensable auxiliary protein that assists Nut2p. Several observations lead to this hypothesis. First, inactivation of a temperature-sensitive Nut2p causes a measurable increase in Swi4p-independent UAS_{GAL}-URS2R-*lacZ* expression despite the presence of wild-type Nut1p. Second, of the two proteins, Nut2p is an essential protein whereas Nut1p is not. Finally, Nut1p appears to assist Nut2p both in the essential function of Nut2p and in regulating the UAS_{GAL}-URS2R-*lacZ* reporter construct. Removal of Nut1p dramatically exacerbates the constitutive reporter phenotype of the *nut2-1* allele. Likewise, in the absence Nut1p, the mutant Nut2p encoded by the *nut2-ts70* allele is unable to sustain cell viability at any temperature, whereas in the presence of Nut1p, it can support viability at 25°C. Thus, Nut1p contributes substantially to the function of Nut2p.

Nut1p, Nut2p, and Ccr4p may comprise a distinctive class of proteins. Our finding that Nut1p and Nut2p function cooperatively suggests that other proteins might cooperate with Nut1p. Therefore, we asked if deletion of *NUT1* could synergize with mutations in genes other than *NUT2* to cause a Nut^- phenotype. From analysis of such mutations, we estimate that at least four such genes exist. An allele of *CCR4* caused a Nut^- phenotype in a *nut1*Δ background, indicating that Ccr4p may function with Nut1p. Ccr4p was previously identified as a regulator of *ADH2* gene expression (15). Its role in gene regulation appears subtle, as the strongest phenotypes were observed on *ADH2* genes that were compromised by *SPT*/*CRE* mutations or by delta insertions. Ccr4p is a component of a large protein complex that includes Caf1p, Dbf2p, Not1p, Not2p, Not3p, and Not4p (16, 30, 31). The Not proteins appear to be involved in transcriptional repression (12). Our finding that Ccr4p functions with Nut1p to negatively regulate UAS_{GAL}-URS2R-*lacZ* is consistent with these studies. Although we have yet to determine the identity of the remaining genes that function like *NUT2*, some may encode other proteins in the Ccr4p complex, and likewise Nut1p and Nut2p might physically interact with the Ccr4p complex. Remarkably, Harashima et al. identified two different two-gene traits, one due to mutations in *BEL3* and *BEL7* and the other due to mutations in *BEL5* and *BEL6*, that affect the repression of *PHO5* at a heterologous genomic locus but not at its native locus (19). Not only is the *nut1 nut2* mutant similarly defective in two genes, but it shares with these two *bel* mutants rough colony morphology, flocculent growth in liquid culture, and elevated expression of artificial, albeit different reporter genes. It remains to be determined if *bel3*, *bel5*, *bel6*, and *bel7* are alleles of *NUT1*, *NUT2*, or *CCR4*.

In summary, the genetic screens described here have identified Sin4p, Nut1p, Nut2p, and Ccr4p as representatives of a distinctive group of proteins. These proteins all appear to negatively regulate transcription from artificial reporters. While Sin4p and Ccr4p are known to regulate endogenous genes, we do not know if Nut1p and Nut2p do. However, Nut2p may be vital to the regulation of endogenous genes, since Nut2p is essential for viability. The disruptive effect of *lacZ* sequences may be revealing a requirement for a function dependent on Sin4p, Nut1p, Nut2p, and Ccr4p. Elucidating this function may contribute to our understanding of the processes that negatively regulate eukaryotic transcription.

ACKNOWLEDGMENTS

We are particularly grateful to Meghan Sharp for isolating the Nut^- mutants and to Sally Horne for characterization of the *NUT2*-like genes, during their rotations in the laboratory, and to Sandy Johnson and Megan Grether for improvements on the manuscript. We thank Marian Carlson, Wolfram Hörz, David Stillman, Madhu Wahi, Richard Young, and their colleagues for generously providing plasmids, and Megan Grether, Carol Gross, Wolfram Hörz, Sandy Johnson, Hay-Oak Park, Sylvia Sanders, Shai Shaham, and Anita Sil for numerous discussions during the course of this work.

This work was supported by NIH research grant AI18738 to I.H., a Howard Hughes predoctoral fellowship to R.K.T., and grants from the Markey Foundation and the Herbert W. Boyer Fund.

ADDENDUM IN PROOF

We have recently learned that Nut2p may be a component of the mediator complex (Claes Gustafsson, personal communication; Young-Joon Kim, personal communication). The mediator complex is required for yeast transcriptional activation in vitro and also contains Sin4p, Rox3p, and Rgr1p (see references 18 and 28).

REFERENCES

1. **Alani, E., L. Cao, and N. Kleckner.** 1987. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. Genetics **116:**541–545.
2. **Almer, A., H. Rudolph, A. Hinnen, and W. Hörz.** 1986. Removal of positioned nucleosomes from the yeast *PHO5* promoter upon *PHO5* induction releases additional upstream activating DNA elements. EMBO J. **5:**2689–2696.
3. **Andrews, B. J., and I. Herskowitz.** 1989. Identification of a DNA binding factor involved in cell-cycle control of the yeast *HO* gene. Cell **57:**21–29.
4. **Andrews, B. J., and I. Herskowitz.** 1989. The yeast Swi4 protein contains a motif present in developmental regulators and is part of a complex involved in cell-cycle-dependent transcription. Nature **342:**830–833.
5. **Andrews, B. J., and L. A. Moore.** 1992. Interaction of the yeast Swi4 and Swi6 cell cycle regulatory proteins *in vitro*. Proc. Natl. Acad. Sci. USA **89:**11852–11856.
6. **Bassett, D. E., Jr., M. S. Boguski, F. Spencer, R. Reeves, M. Goebl, and P. Hieter.** 1995. Comparative genomics, genome cross-referencing and XREFdb. Trends Genet. **11:**372–373.
7. **Bobola, N., R. P. Jansen, T. H. Shin, and K. Nasmyth.** 1996. Asymmetric accumulation of Ash1p in postanaphase nuclei depends on a myosin and restricts yeast mating-type switching to mother cells. Cell **84:**699–709.
8. **Bortvin, A., and F. Winston.** 1996. Evidence that Spt6p controls chromatin structure by a direct interaction with histones. Science **272:**1473–1476.
9. **Breeden, L., and K. Nasmyth.** 1987. Cell cycle control of the yeast *HO* gene: cis- and trans-acting regulators. Cell **48:**389–397.
10. **Chen, S., R. W. West, Jr., S. L. Johnson, H. Gans, B. Kruger, and J. Ma.** 1993. TSF3, a global regulatory protein that silences transcription of yeast *GAL* genes, also mediates repression by α2 repressor and is identical to SIN4. Mol. Cell. Biol. **13:**831–840.
11. **Clark-Adams, C. D., D. Norris, M. A. Osley, J. S. Fassler, and F. Winston.** 1988. Changes in histone gene dosage alter transcription in yeast. Genes Dev. **2:**150–159.
12. **Collart, M. A., and K. Struhl.** 1994. *NOT1* (*CDC39*), *NOT2* (*CDC36*), *NOT3*, and *NOT4* encode a global-negative regulator of transcription that differentially affects TATA-element utilization. Genes Dev. **8:**525–537.
13. **Covitz, P. A., W. Song, and A. P. Mitchell.** 1994. Requirement for RGR1 and SIN4 in RME1-dependent repression in *Saccharomyces cerevisiae*. Genetics **138:**577–586.
14. **Cross, F. R., M. Hoek, J. D. McKinney, and A. H. Tinkelenberg.** 1994. Role of Swi4 in cell cycle regulation of *CLN2* expression. Mol. Cell. Biol. **14:**4779–4787.
15. **Denis, C. L., and T. Malvar.** 1990. The *CCR4* gene from *Saccharomyces cerevisiae* is required for both nonfermentative and spt-mediated gene expression. Genetics **124:**283–291.
16. **Draper, M. P., C. Salvadore, and C. L. Denis.** 1995. Identification of a mouse protein whose homolog in *Saccharomyces cerevisiae* is a component of the CCR4 transcriptional regulatory complex. Mol. Cell. Biol. **15:**3487–3495.

17. **Eisenmann, D. M., C. Dollard, and F. Winston.** 1989. *SPT15*, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation in vivo. Cell **58:**1183–1191.
18. **Gustafsson, C. M., L. C. Myers, Y. Li, M. J. Redd, M. Lui, H. Erdjument-Bromage, P. Tempst, and R. D. Kornberg.** 1997. Identification of Rox3 as a component of mediator and RNA polymerase II holoenzyme. J. Biol. Chem. **272:**48–50.
19. **Harashima, S., T. Mizuno, H. Mabuchi, S. Yoshimitsu, R. Ramesh, M. Hasebe, A. Tanaka, and Y. Oshima.** 1995. Mutations causing high basal level transcription that is independent of transcriptional activators but dependent on chromosomal position in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **247:**716–725.
20. **Hengartner, C. J., C. M. Thompson, J. Zhang, D. M. Chao, S. M. Liao, A. J. Koleske, S. Okamura, and R. A. Young.** 1995. Association of an activator with an RNA polymerase II holoenzyme. Genes Dev. **9:**897–910.
21. **Herschbach, B. M., and A. D. Johnson.** 1993. Transcriptional repression in eukaryotes. Annu. Rev. Cell Biol. **9:**479–509.
21a. **Horne, S., and R. K. Tabtiang.** Unpublished data.
21b. **Hörz, W.** Personal communication.
22. **Jensen, R., G. F. Sprague, Jr., and I. Herskowitz.** 1983. Regulation of yeast mating-type interconversion: feedback control of *HO* gene expression by the mating-type locus. Proc. Natl. Acad. Sci. USA **80:**3035–3039.
23. **Jiang, Y. W., P. R. Dohrmann, and D. J. Stillman.** 1995. Genetic and physical interactions between yeast RGR1 and SIN4 in chromatin organization and transcriptional regulation. Genetics **140:**47–54.
24. **Jiang, Y. W., and D. J. Stillman.** 1992. Involvement of the SIN4 global transcriptional regulator in the chromatin structure of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:**4503–4514.
25. **Kruger, W., C. L. Peterson, A. Sil, C. Coburn, G. Arents, E. N. Moudrianakis, and I. Herskowitz.** 1995. Amino acid substitutions in the structured domains of histones H3 and H4 partially relieve the requirement of the yeast SWI/SNF complex for transcription. Genes Dev. **9:**2770–2779.
26. **Kuchin, S., P. Yeghiayan, and M. Carlson.** 1995. Cyclin-dependent protein kinase and cyclin homologs SSN3 and SSN8 contribute to transcriptional control in yeast. Proc. Natl. Acad. Sci. USA **92:**4006–4010.
27. **Kunkel, T. A.** 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82:**488–492.
28. **Li, Y., S. Bjorklund, Y. W. Jiang, Y. J. Kim, W. S. Lane, D. J. Stillman, and R. D. Kornberg.** 1995. Yeast global transcriptional regulators Sin4 and Rgr1 are components of mediator complex/RNA polymerase II holoenzyme. Proc. Natl. Acad. Sci. USA **92:**10864–10868.
29. **Liao, S. M., J. Zhang, D. A. Jeffery, A. J. Koleske, C. M. Thompson, D. M. Chao, M. Viljoen, H. J. van Vuuren, and R. A. Young.** 1995. A kinase-cyclin pair in the RNA polymerase II holoenzyme. Nature **374:**193–196.
30. **Liu, H., V. Badarinarayana, D. C. Audino, J. Rappsilber, M. Mann, and C. L. Denis.** 1998. The NOT proteins are part of the CCR4 transcriptional complex and affect gene expression both positively and negatively. EMBO J. **17:**1096–1106.
31. **Liu, H., J. H. Toyn, Y.-C. Chiang, M. P. Draper, L. H. Johnston, and C. L. Denis.** 1997. DBF2, a cell cycle-regulated protein kinase, is physically and functionally associated with the CCR4 transcriptional regulatory complex. EMBO J. **16:**5289–5298.
32. **Lycan, D., G. Mikesell, M. Bunger, and L. Breeden.** 1994. Differential effects of Cdc68 on cell cycle-regulated promoters in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:**7455–7465.
33. **Macatee, T., Y. W. Jiang, D. J. Stillman, and S. Y. Roth.** 1997. Global alterations in chromatin accessibility associated with loss of SIN4 function. Nucleic Acids Res. **25:**1240–1247.
34. **Muhlrad, D., R. Hunter, and R. Parker.** 1992. A rapid method for localized mutagenesis of yeast genes. Yeast **8:**79–82.
35. **Nasmyth, K.** 1983. Molecular analysis of a cell lineage. Nature **302:**670–676.
36. **Nasmyth, K.** 1985. At least 1400 base pairs of 5′-flanking DNA is required for the correct expression of the *HO* gene in yeast. Cell **42:**213–223.
37. **Nasmyth, K.** 1985. A repetitive DNA sequence that confers cell-cycle START (CDC28)-dependent transcription of the *HO* gene in yeast. Cell **42:**225–235.
38. **Nasmyth, K.** 1987. The determination of mother cell-specific mating type switching in yeast by a specific regulator of *HO* transcription. EMBO J. **6:**243–248.
39. **Nasmyth, K., and L. Dirick.** 1991. The role of SW14 and SW16 in the activity of G1 cyclins in yeast. Cell **66:**995–1013.
40. **Nasmyth, K., D. Stillman, and D. Kipling.** 1987. Both positive and negative regulators of *HO* transcription are required for mother-cell-specific mating-type switching in yeast. Cell **48:**579–587.
41. **Nonet, M. L., and R. A. Young.** 1989. Intragenic and extragenic suppressors of mutations in the heptapeptide repeat domain of *Saccharomyces cerevisiae* RNA polymerase II. Genetics **123:**715–724.
42. **Ogas, J., B. J. Andrews, and I. Herskowitz.** 1991. Transcriptional activation of *CLN1*, *CLN2*, and a putative new G1 cycline (*HCS26*) by SWI4, a positive regulator of G1-specific transcription. Cell **66:**1015–1026.
43. **Primig, M., S. Sockanathan, H. Auer, and K. Nasmyth.** 1992. Anatomy of a transcription factor important for the start of the cell cycle in *Saccharomyces cerevisiae*. Nature **358:**593–597.
44. **Rose, M. D., and J. R. Broach.** 1991. Cloning genes by complementation in yeast, p. 195–230. *In* C. Guthrie, and G. R. Fink (ed.), Guide to yeast genetics and molecular biology, vol. 194. Academic Press, Inc., San Diego, Calif.
45. **Rose, M. D., F. Winston, and P. Hieter.** 1990. Methods in yeast genetics: a laboratory course manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
46. **Rosenblum-Vos, L. S., L. Rhodes, C. C. Evangelista, Jr., K. A. Boayke, and R. S. Zitomer.** 1991. The *ROX3* gene encodes an essential nuclear protein involved in *CYC7* gene expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**5639–5647.
47. **Rothstein, R.** 1991. Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast, p. 281–301. *In* C. Guthrie and G. R. Fink (ed.), Guide to yeast genetics and molecular biology, vol. 194. Academic Press, Inc., San Diego, Calif.
48. **Russell, D. W., R. Jensen, M. J. Zoller, J. Burke, B. Errede, M. Smith, and I. Herskowitz.** 1986. Structure of the *Saccharomyces cerevisiae HO* gene and analysis of its upstream regulatory region. Mol. Cell. Biol. **6:**4281–4294.
49. **Sakai, A., Y. Shimizu, S. Kondou, T. Chibazakura, and F. Hishinuma.** 1990. Structure and molecular analysis of *RGR1*, a gene required for glucose repression of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**4130–4138.
50. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
51. **Sidorova, J., and L. Breeden.** 1993. Analysis of the SWI4/SWI6 protein complex, which directs G_1/S-specific transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **13:**1069–1077.
52. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
53. **Sil, A., and I. Herskowitz.** 1996. Identification of asymmetrically localized determinant, Ash1p, required for lineage-specific transcription of the yeast *HO* gene. Cell **84:**711–722.
54. **Song, W., I. Treich, N. Qian, S. Kuchin, and M. Carlson.** 1996. *SSN* genes that affect transcriptional repression in *Saccharomyces cerevisiae* encode SIN4, ROX3, and SRB proteins associated with RNA polymerase II. Mol. Cell. Biol. **16:**115–120.
55. **Stern, M., R. Jensen, and I. Herskowitz.** 1984. Five *SWI* genes are required for expression of the *HO* gene in yeast. J. Mol. Biol. **178:**853–868.
56. **Sternberg, P. W., M. J. Stern, I. Clark, and I. Herskowitz.** 1987. Activation of the yeast *HO* gene by release from multiple negative controls. Cell **48:**567–577.
57. **Straka, C., and W. Hörz.** 1991. A functional role for nucleosomes in the repression of a yeast promoter. EMBO J. **10:**361–368.
58. **Strich, R., M. R. Slater, and R. E. Esposito.** 1989. Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast. Proc. Natl. Acad. Sci. USA **86:**10018–10022.
58a. **Tabtiang, R. K.** Unpublished data.
59. **Thompson, C. M., A. J. Koleske, D. M. Chao, and R. A. Young.** 1993. A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell **73:**1361–1375.
60. **Vallier, L. G., and M. Carlson.** 1994. Synergistic release from glucose repression by *mig1* and *ssn* mutations in *Saccharomyces cerevisiae*. Genetics **137:**49–54.
61. **Wahi, M., and A. D. Johnson.** 1995. Identification of genes required for α2 repression in *Saccharomyces cerevisiae*. Genetics **140:**79–90.
62. **Winston, F., D. T. Chaleff, B. Valent, and G. R. Fink.** 1984. Mutations affecting Ty-mediated expression of the *HIS4* gene of *Saccharomyces cerevisiae*. Genetics **107:**179–197.