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Structure and Expression of the *SNF1* Gene of *Saccharomyces cerevisiae*

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The *SNF1* gene of *Saccharomyces cerevisiae* is essential for normal regulation of gene expression by glucose repression. A functional *SNF1* gene product is required to derepress many glucose-repressible genes in response to conditions of low external glucose. In the case of the *SUC2* structural gene for invertase, *SNF1* acts at the RNA level. We have reported the isolation of a cloned gene that complements the *snf1* defect in *S. cerevisiae* and that is homologous to DNA at the *SNF1* locus (J. L. Celenza and M. Carlson, Mol. Cell. Biol. **4**:49–53, 1984). In this work we identified a 2.4-kilobase polyadenylate-containing RNA encoded by the *SNF1* gene and showed that its level is neither regulated by glucose repression nor dependent on a functional *SNF1* product. The position of the *SNF1* RNA relative to the cloned DNA was mapped, and the direction of transcription was determined. The cloned DNA was used to disrupt the *SNF1* gene at its chromosomal locus. Gene disruption resulted in a Snf1$^-$ phenotype, thereby proving that the cloned gene is the *SNF1* gene and showing that the phenotype of a true null mutation is indistinguishable from that of previously isolated *snf1* mutations.

The *SNF1* gene (sucrose nonfermenting) is required for growth on a variety of fermentable and nonfermentable carbon sources. The *SNF1* gene was first identified by mutational analysis as a gene required for sucrose fermentation. Eight recessive *snf1* mutant alleles have been isolated and all are pleiotropic (6; L. Neigeborn and M. Carlson, unpublished data). Utilization of all the affected carbon sources is regulated by glucose repression (6). The *snf1* mutations also confer an inability to sporulate that could result from a defect in glucose regulation (6). The failure of *snf1* mutants to utilize sucrose stems from a defect in derepressing expression of the structural gene for invertase at the RNA level; the RNA encoding the secreted invertase enzyme, which hydrolyzes sucrose, is not produced in *snf1* mutant cells grown under derepressing conditions (5). Although the molecular basis of the other phenotypes of the *snf1* mutation has not yet been investigated, the genetic and biochemical evidence suggests that the *SNF1* gene product acts positively to derepress expression of glucose-repressible genes in response to low glucose concentrations in the environment. The pleiotropy of *snf1* mutations suggests that *SNF1* plays a central regulatory role. As a first step toward identifying the *SNF1* gene product and elucidating its role in glucose repression, we undertook a study of the structure and expression of the *SNF1* gene.

In the accompanying paper, we reported the isolation of a cloned DNA segment that complements a *snf1* mutation in *Saccharomyces cerevisiae* and that is homologous to sequences at the *SNF1* locus on chromosome IV (8). These properties strongly suggest that the cloned DNA contains the *SNF1* gene. We therefore proceeded to characterize this gene and its expression. We identified the *SNF1* RNA, investigated the regulation of its synthesis, and mapped the transcriptional unit relative to the cloned DNA. Locating the gene enabled us to use the cloned DNA to disrupt the gene at its chromosomal locus. In these studies, we identified the phenotype of a null allele of *snf1* and proved that the cloned gene is *SNF1*.

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MATERIALS AND METHODS

Strains, genetic methods, and yeast transformation. *S. cerevisiae* strains and genotypes are listed in Table 1. Standard yeast genetic procedures were followed (16) as described in the accompanying paper (8). Minimal media containing different carbon sources were prepared by the method of Sherman et al. (16), except that the indicated carbon source was substituted for glucose. Yeast cells were transformed by the method of Ito et al. (10) with 0.1 M lithium acetate or by the method of Hinnen et al. (9).

Preparation and analysis of DNA. Plasmid DNAs and yeast genomic DNAs were prepared as described previously (8). Analysis of DNAs by restriction enzyme digestion and agarose gel electrophoresis was carried out as before (8). Gel transfer hybridization (18) was carried out by the method of Wahl et al. (20) with dextran sulfate and radioactively labeled probes prepared by nick translation (15).

Construction of subclones. To subclone restriction fragments containing portions of the 3.5-kilobase (kb) complementing region, plasmid pCN1 DNA (8) was digested with *Bam*HI and partially digested with *Bgl*II and then extracted with phenol and precipitated with ethanol. Vector DNAs pBR322 (3), YIp5 (4), and YEp24 (4) were cleaved with *Bam*HI, treated with calf intestinal phosphatase (Boehringer Mannheim), extracted with phenol, and precipitated with ethanol. The pCN1 DNA fragments were ligated to each of these vectors, and the ligated DNAs were used to transform *Escherichia coli* HB101 as described previously (8). Plasmids pCC8 and pCC10 were derived from pBR322; pCN8 and pCN9 were derived from YIp5; and pCE8, pCE9, and pCE10 were derived from YEp24 (Fig. 1).

Plasmid pCN11 was constructed by digesting pCC10 simultaneously with *Sau*3AI, *Bgl*I, and *Hin*dIII. The 0.65-kb *Sau*3AI fragment (Fig. 1) (one end of which was a *Bgl*II site) was purified by electrophoresing the digestion products on an agarose gel in 40 mM Tris acetate and 2 mM EDTA (pH 7.5) and by isolating the DNA from the excised band with glass powder (19). *Bgl*I and *Hin*dIII cleaved two *Sau*3AI fragments which would otherwise have comigrated with the

TABLE 1. *S. cerevisiae* strains

Strain	Genotype[a]	Source or reference
MCY405	*MATα ura3-52 SUC2⁺ gal2*	This work
MCY419	*MATα ura3-52 snf1-31 lys2-801 his4-539 SUC2⁺*	8
MCY643	*MATa ura3-52 lys2-801 his4-539 SUC2⁺* / *MATα ura3-52 lys2-801 + SUC2⁺*	This work
DBY782	*MATα ade2-101 SUC2⁺ gal2*	5
DBY934	*MATa snf1-28 his4-619 SUC2⁺ gal2*	5

[a] All strains are isogenic or congenic to S288C.

desired fragment. The 0.65-kb fragment was ligated into the *Bam*HI site of the YIp5 vector prepared above. The ligated DNA was then used to transform *E. coli* HB101 to ampicillin resistance, and pCN11 was recovered. The orientation of the inserted fragment with respect to the vector was determined by digestion with *Hin*cII, which cuts asymmetrically in the cloned fragment.

Preparation and analysis of RNA. Polyadenylate [poly(A)]-containing RNAs were prepared from glucose-repressed and -derepressed cells as described previously (5). For gel transfer hybridization, RNAs were treated with glyoxal and dimethyl sulfoxide (12), separated by agarose gel electrophoresis, and transferred from the gel by the method of Alwine et al. (1) to diazotized paper as described before (5). Hybridization was carried out as described previously (1), except that filters were washed in 15 mM NaCl, 1.5 mM sodium citrate, and 0.1% sodium dodecyl sulfate at 50°C.

S1 mapping. S1 mapping was carried out with end-labeled DNA as described before (7), by the method of Berk and Sharp (2) as modified by Weaver and Weissman (21). Hybridization was carried out at 46°C for 3 h. Samples were analyzed by electrophoresis in 1.5% alkaline agarose (11), and the dried gel was allowed to expose Kodak XR-5 film at −70°C with DuPont Lightning Plus screens.

RESULTS

Location of the *SNF1* gene in the cloned sequence. Previous results localized the putative *SNF1* gene to a 3.5-kb region common to all recombinant plasmids capable of complementing the *snf1* defect (8). To define further the location of the *SNF1* gene, we subcloned three restriction fragments from this region into the episomal vector YEp24 (4) and recovered plasmids pCE8, pCE9, and pCE10 as described above. Plasmids pCE8 and pCE10 each contain part of the 3.5-kb region, and pCE9 contains almost the entire 3.5-kb region (Fig. 1). Together, plasmids pCE8 and pCE10 contain all the sequences present in pCE9. The vector YEp24 contains the *URA3* gene and can replicate autonomously in *S. cerevisiae*. The ability of each subclone to complement *snf1* was tested by using each DNA to transform (10) strain MCY419 (*snf1 ura3*) to uracil prototrophy. Six independent Ura⁺ transformants from each experiment were examined for the Snf1 phenotype. All six strains transformed with plasmid pCE9 exhibited a Snf1⁺ phenotype with respect to growth on sucrose. This result was expected because pCE9 contains almost the entire 3.5-kb common region. In contrast, transformants carrying pCE8 and pCE10 showed a Snf1⁻ phenotype, indicating that these subclones are unable to complement *snf1*. Because pCE8 and pCE10 together contain all the sequences present in pCE9 and yet neither complements *snf1*, we concluded that the *Bgl*II site forming the boundary between the two subcloned sequences lies within the *SNF1* gene.

Identification of the *SNF1* RNA. Plasmids pCE8 and pCE10 appeared to each contain part of the *SNF1* gene, so it seemed likely that the *SNF1* RNA would be encoded by sequences present on both plasmids. We therefore attempted to identify an RNA homologous to sequences on both plasmids. Because the *SNF1* gene has a role in regulation of gene expression in response to glucose concentration, the possibility existed that the *SNF1* RNA would be expressed only in glucose-repressed or -derepressed cells. We therefore examined poly(A)-containing RNA from cells grown under both conditions, using the method of RNA gel transfer hybridization (1). The RNAs were electrophoresed on an agarose gel, transferred to diazotized paper, and hybridized with radioactively labeled probes. Probes were prepared from plasmids pCC8 and pCC10, which contain the same subcloned fragments as pCE8 and pCE10, respectively; pCC8 and pCC10 are derived from pBR322 and therefore contain no other yeast DNA sequences (Fig. 1). A 2.4-kb RNA was detected in both RNA samples with both probes (data not shown) (see Fig. 2). In addition, a 0.9-kb RNA was detected with the pCC8 probe; further experiments showed that a restriction fragment extending 1.1 kb to the left of the *Eco*RI site in pCC8 was homologous only to the 0.9-kb RNA (data not shown). These data suggest that the 2.4-kb RNA is encoded by *SNF1*; experiments described below confirm this identification.

Level of *SNF1* RNA is not regulated by glucose repression or dependent on a functional *SNF1* gene product. The presence of the 2.4-kb *SNF1* RNA in both glucose-repressed and -derepressed cells suggested that the level of *SNF1* RNA may not be regulated by glucose. Another experiment was undertaken to address this issue. Poly(A)-containing RNAs from *SNF1*⁺ cells grown under both conditions were analyzed by RNA gel transfer hybridization as described above. For this experiment, RNAs were detected by hybridization with radioactively labeled plasmid pCN8 DNA. This plasmid was constructed by inserting the same *Bgl*II fragment that was subcloned in pCE8 and pCC8 into the vector YIp5 (4; Fig. 1). Because YIp5 contains the *URA3* gene, this probe hybridized to the 2.4-kb *SNF1* RNA, the 0.9-kb *URA3* RNA, and the unidentified 0.9-kb RNA from the *SNF1* locus described above (Fig. 2). To confirm that RNAs known to be expressed in repressed and derepressed cells were represented in the appropriate samples, the *SUC2* mRNAs were also detected on this filter. As expected, the 1.9- and 1.8-kb

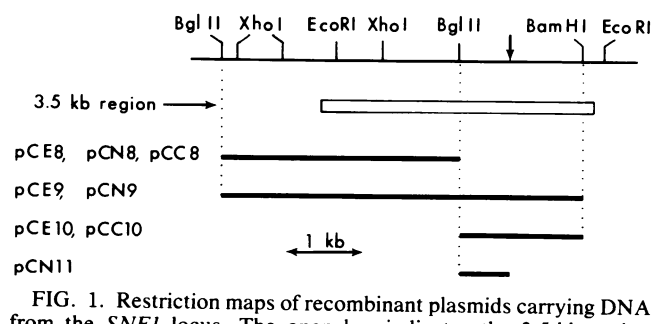

FIG. 1. Restriction maps of recombinant plasmids carrying DNA from the *SNF1* locus. The open bar indicates the 3.5-kb region common to plasmids that complement a *snf1* mutation (8). The solid bars represent the yeast DNA fragments subcloned in the indicated plasmids as described in the text. Plasmids are named according to the vector used in their construction; pCE, pCN, and pCC refer to the vectors YEp24, YIp5, and pBR322, respectively. The arrow indicates the *Sau*3AI site used in constructing pCN11; other *Sau*3AI sites are not shown, except for those which are also *Bgl*II or *Bam*HI sites.

SUC2 mRNAs were present in the RNA from glucose-derepressed cells, and only the 1.8-kb *SUC2* mRNA was found in the RNA from repressed cells (5). The level of *SNF1* RNA appeared equivalent in the two samples; although a higher level of hybridization was observed in the sample from repressed cells, more of this RNA was loaded on the gel and the 0.9- and 1.8-kb RNAs also showed corresponding quantitative changes. The 1.8-kb *SUC2* RNA, the *URA3* RNA, and the unidentified 0.9-kb RNA are not regulated by glucose (5; unpublished data).

We also investigated the possibility that a functional *SNF1* gene product is required for regulation of *SNF1* gene expression. Poly(A)-containing RNA was prepared from a *snf1* mutant strain, DBY934, grown under glucose-repressing and -derepressing conditions, and was analyzed in the experiment described above. The 2.4-kb *SNF1* RNA was present at the same level in both samples (Fig. 2, lanes c and d); in this case, approximately equal amounts of RNA were loaded in the two lanes, and the 0.9- and 1.8-kb RNAs were labeled with equal intensity. The 1.9-kb *SUC2* mRNA is not produced by *snf1* mutants. The mutation at *SNF1* (the *snf1-28* allele) does not appear to affect expression of the *SNF1* RNA.

Map of the *SNF1* transcriptional unit. The map position of

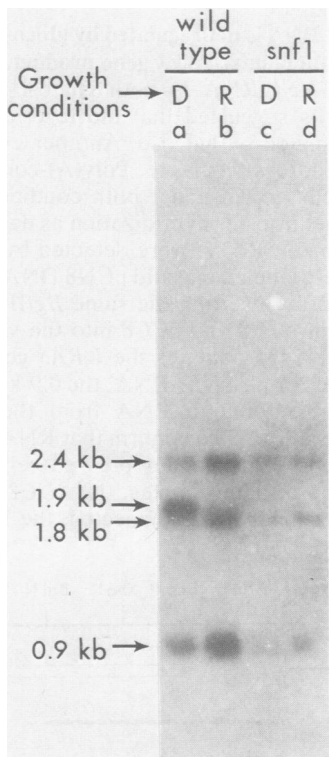

FIG. 2. *SNF1* RNA is present in glucose-repressed and -derepressed cells. Poly(A)-containing RNA was prepared from glucose-repressed and -derepressed yeast strains and analyzed by gel-transfer hybridization as described in the text. The 0.9- and 2.4-kb RNAs were detected by hybridization with the pCN8 probe, and the 1.8- and 1.9-kb *SUC2* mRNAs were detected with the pRB118 probe (5). Sizes were determined by comparison with rRNAs (14), the *SUC2* mRNAs (5), and glyoxal-treated DNA standards. RNA samples were prepared from cells as follows: (lane a) wild-type DBY782 grown in glucose-derepressing conditions (D) as described in the text; (lane b) DBY782 grown in glucose-repressing conditions (R); (lane c) DBY934 (*snf1*) grown in derepressing conditions; (lane d) DBY934 grown in repressing conditions.

the *SNF1* RNA and its direction of transcription relative to the cloned DNA were determined by the method of S1 nuclease protection (2). Plasmid pCN9 DNA was digested with endonuclease *Bgl*II, which cleaves at a unique site within the *SNF1* gene (see Fig. 1), and the 5′ ends at the restriction site were labeled with ^{32}P as described above. The labeled DNA was denatured and allowed to hybridize to poly(A)-containing RNA; the samples were then treated with S1 nuclease to degrade single-stranded DNA that was not protected by hybrid formation with RNA. The protected DNA fragments were analyzed by electrophoresis on an alkaline-agarose gel, and the end-labeled DNA fragments were detected by autoradiography. A 0.8-kb fragment was detected in this experiment (Fig. 3, lane e). The size of the protected fragment corresponds to the size of the contiguous RNA coding sequence extending from the labeled *Bgl*II site toward the 5′ end of the RNA.

The orientation of the 5′ end of the RNA relative to the restriction map of the cloned gene could not be determined from these data. For this purpose, we took advantage of an *Ava*II site located 0.35 kb from the *Bgl*II site. If the 5′ end of the RNA mapped on the same side of the *Bgl*II site as the *Ava*II site, digestion of the 5′-end-labeled probe with *Ava*II before hybridization would result in protection of a 0.35-kb fragment rather than a 0.8-kb fragment. If the 5′ end of the RNA mapped on the opposite side of the *Bgl*II site, digestion with *Ava*II would not alter the size of the 0.8-kb, protected fragment. The experiment was carried out with *Ava*II-digested probe, and no 0.8-kb fragment was protected; instead, a 0.35-kb fragment was detected (Fig. 3, lane c). The diagram in Fig. 3 shows the correct orientation of the transcriptional unit.

To map the extent of contiguous RNA coding sequences from the *Bgl*II site toward the 3′ end of the RNA, an analogous experiment was carried out with pCE5 DNA which was 3′ end labeled at the *Bgl*II site as the probe. A 1.55-kb fragment was protected by RNA (Fig. 4, lane d). The orientation of the 3′ end with respect to the *Bgl*II site was confirmed by digesting the probe with *Ava*II before hybridization with RNA. The only *Ava*II site in pCE5 within 1.55 kb of the *Bgl*II site is the *Ava*II site 0.35 kb toward the 5′ end of the RNA. As expected, *Ava*II digestion of the probe did not affect the size of the 1.55-kb, protected fragment (Fig. 4, lane c).

The total extent of the contiguous sequence protected by RNA in these experiments is 2.35 kb (the sum of the two protected fragments). The size of the *SNF1* RNA identified by RNA gel transfer hybridization was 2.4 kb. This close agreement in size indicates that the *SNF1* gene does not contain an intron in the central portion of the coding sequence; however, an intron very close to the 5′ or 3′ end of the transcriptional unit could possibly have escaped detection.

Disruption of the *SNF1* gene at its chromosomal locus. Having mapped the position of the *SNF1* gene on the cloned DNA sequence, we were able to use the cloned DNA to disrupt the *SNF1* gene at its chromosomal locus. Such an experiment serves two purposes. First, our cloned gene is proved to be *SNF1* if disruption of the chromosomal copy of the cloned gene produces a Snf1$^-$ phenotype. Second, the phenotype of a true null mutation is identified; the eight *snf1* alleles we isolated are likely to be point mutations and none is known to be a nonsense mutation (6; L. Neigeborn and M. Carlson, unpublished data).

To disrupt the *SNF1* gene, we applied the method of Shortle et al. (17) (Fig. 5). First, the 0.65-kb *Sau*3AI frag-

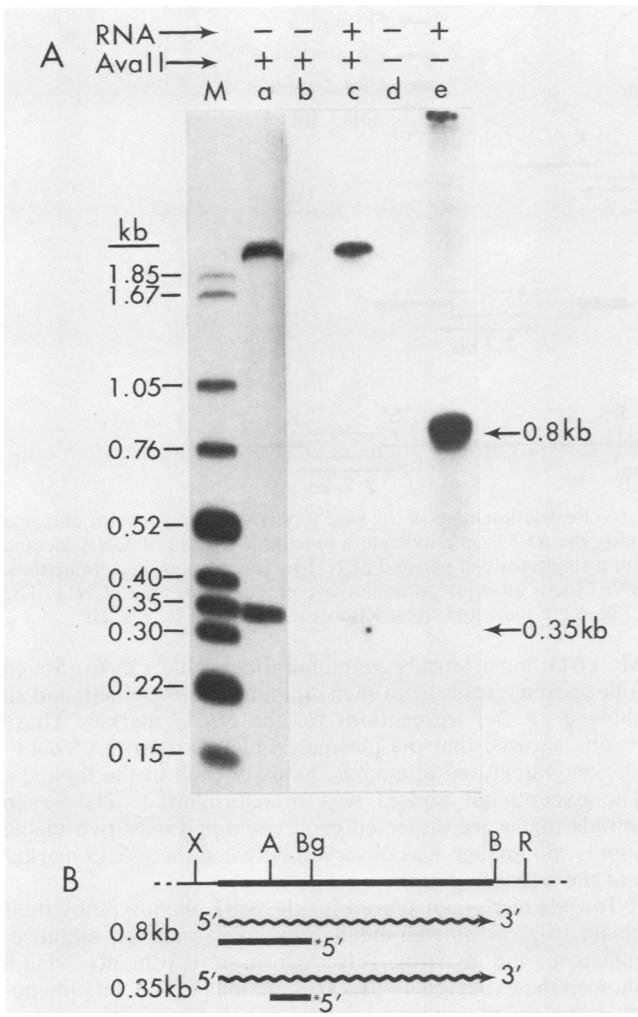

FIG. 3. (A) Mapping the 5′ end of the *SNF1* RNA. S1 nuclease protection experiments were carried out as described in the text with a probe prepared by 5′ end labeling pCN9 DNA at the *Bgl*II site. Where *Ava*II digestion is indicated (+), the probe was digested with *Ava*II before hybridization. Protected fragments were separated by electrophoresis and detected by autoradiography. DNA fragments produced by digesting the probe with *Ava*II (lane a). DNA fragments protected by poly(A)-containing RNA prepared from glucose-repressed cells of strain DBY782 (*SNF1*$^+$) with intact probe (lane e) and *Ava*II-digested probe (lane c). A 0.8-kb fragment of the intact probe is protected. This 0.8-kb fragment was not observed when the *Ava*II-digested probe was used. Instead, a 0.35-kb fragment was detected, although weakly; the appearance of this fragment could result from both protection by RNA and renaturation of the probe DNA. The upper band in each lane is renatured probe DNA (a 2.0-kb fragment in the case of *Ava*II-digested probe [lane c]). Control samples showing that in the absence of yeast RNA, there is no protection of labeled DNA (lanes b and d); *E. coli* tRNA was added instead of yeast RNA. Marker (lane M). (B) Protection of the 0.8- and 0.35-kb fragments. A restriction map of the *SNF1* transcriptional unit (solid bar) is shown. The direction of transcription is indicated by the polarity of the *SNF1* RNA (wavy line). The asterisks indicate ^{32}P-labeled 5′ ends. Restriction sites: X, *Xho*I; A, *Ava*II; Bg, *Bgl*II; B, *Bam*HI; R, *Eco*RI.

ment that mapped within the *SNF1* gene was subcloned into the *Bam*HI site of integrating vector YIp5 (4), which carries the yeast *URA3* gene (Fig. 1). Then, the resulting plasmid, pCN11, was used to transform (9) haploid strain MCY405 (*ura3 SNF1*$^+$) and diploid strain MCY643 (*ura3/ura3 SNF1*$^+$/*SNF1*$^+$) to uracil prototrophy. Integration of this plasmid at the *SNF1* locus by homologous recombination creates two incomplete copies of the *SNF1* gene surrounding the vector sequence (Fig. 5); integration can also occur at the *URA3* locus. A diploid strain was included because of the possibility that creation of a null allele of *snf1* would prove lethal in a haploid strain.

Only six transformants of MCY405 were recovered; all showed a Snf1$^+$ phenotype and were not studied further. Thirteen diploid transformants were recovered and seven were analyzed to determine the site of plasmid integration by

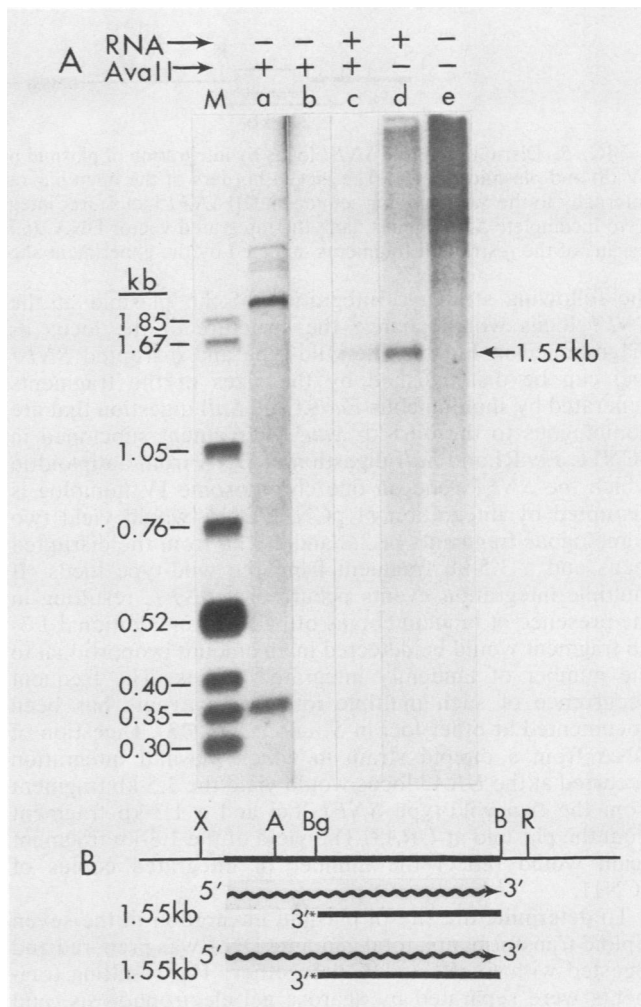

FIG. 4. (A) Mapping the 3′ end of the *SNF1* RNA. S1 nuclease protection mapping was performed with a probe prepared by 3′ end labeling pCE5 DNA at the *Bgl*II site. The probe was digested with *Ava*II before hybridization where indicated (+). Protected fragments were resolved by electrophoresis and detected by autoradiography. DNA fragments generated by *Ava*II digestion of the probe (lane a). DNA fragments protected by poly(A)-containing RNA prepared from glucose-repressed DBY782 cells with intact probe (lane d) and *Ava*II-digested probe (lane c). In both cases, a 1.55-kb fragment was protected. Control samples showing no protection of labeled DNA when *E. coli* tRNA was substituted for yeast RNA (lanes b and e). Marker (M). (B) Origin of the protected 1.55-kb fragments. A map of the *SNF1* transcriptional unit (solid bar) is shown, and the *SNF1* RNA is represented by the wavy line. The asterisks indicate ^{32}P-labeled 3′ ends. Restriction sites: X, *Xho*I; A, *Ava*II; Bg, *Bgl*II; B, *Bam*HI; R, *Eco*RI.

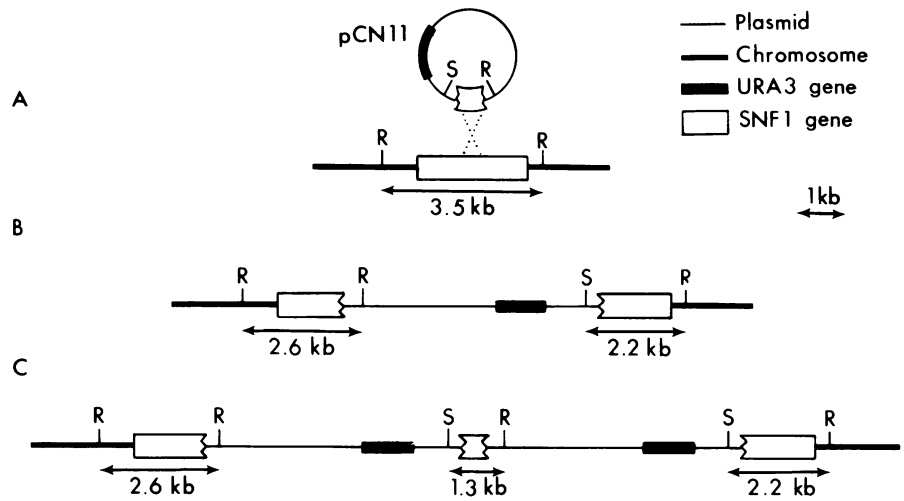

FIG. 5. Disruption of the *SNF1* locus by integration of plasmid pCN11. (A) Restriction maps of the wild-type *SNF1* locus on chromosome IV (8) and plasmid pCN11. The jagged borders of the open bar representing the *SNF1* gene indicate a junction with vector DNA located internally to the *SNF1* coding sequence. (B) *SNF1* locus after integration of a single copy of plasmid pCN11 by homologous recombination. Two incomplete *SNF1* genes flank the integrated vector DNA. (C) The *SNF1* locus after integration of two copies of plasmid pCN11. The origins of the restriction fragments detected by the experiment shown in Fig. 6 are indicated. Restriction sites: S, *Sal*I; R, *Eco*RI.

the following strategy. Integration of the plasmid at the *SNF1* locus would change the structure of the locus as diagrammed in Fig. 5. The wild-type and disrupted *SNF1* loci can be distinguished by the sizes of the fragments generated by simultaneous *Eco*RI and *Sal*I digestion that are homologous to the 0.65-kb *Sau*3AI fragment subcloned in pCN11. *Eco*RI and *Sal*I digestion of DNA from a diploid in which the *SNF1* gene on one chromosome IV homolog is disrupted by integration of pCN11 DNA would yield two homologous fragments of 2.2 and 2.6 kb from the disrupted locus and a 3.5-kb fragment from the wild-type locus. If multiple integration events occurred at *SNF1*, resulting in the presence of tandem copies of pCN11, an additional 1.3-kb fragment would be detected in an amount proportional to the number of tandemly integrated copies. The frequent occurrence of such multiple integration events has been documented at other loci in *S. cerevisiae* (13). Digestion of DNA from a diploid strain in which plasmid integration occurred at the *URA3* locus would yield the 3.5-kb fragment from the two wild-type *SNF1* loci and a 1.3-kb fragment from the plasmid at *URA3*. The yield of the 1.3-kb fragment again would reflect the number of integrated copies of pCN11.

To determine the site of plasmid integration in the seven diploid transformants, total genomic DNA was prepared and digested with *Eco*RI and *Sal*I together. The resulting fragments were separated by agarose gel electrophoresis, and fragments homologous to the 0.65-kb *Sau*3AI fragment from the *SNF1* gene were detected by gel transfer hybridization (Fig. 6). Diploid transformants 1, 2, 3, 6, and 12 gave rise to the 2.2-, 2.6-, and 3.5-kb fragments expected from a strain carrying one disrupted *SNF1* gene and one wild-type gene. Transformants 1, 2, and 6 appeared to have multiple integrated copies of pCN11, presumably also at the *SNF1* locus, as judged by the presence of the 1.3-kb fragment. Transformants 7 and 8 did not produce the fragments characteristic of a *SNF1* gene disruption; thus, plasmid integration occurred at another locus, most likely *URA3*.

These diploid transformants were induced to sporulate, and four-spored asci were dissected for tetrad analysis. The spore viability for the transformants was, with one exception (see below), the same as observed for the parent diploid MCY643; most tetrads contained four viable spores. Seven four-spored tetrads from each diploid were examined, and all showed 2+:2− segregations for the *URA3* marker. These results showed that the plasmid, which carried the *URA3*$^+$ marker, integrated at a single locus in each of the diploids. The exceptional diploid was transformant 2. The seven tetrads that were dissected each contained only two viable spores; no linkage was observed between the *URA3* marker and the lethal mutation.

Tetrads were then scored for the Snf1 phenotype by their ability to grow on rich media containing sucrose, raffinose, galactose, and glycerol. Tetrads from transformants 7 and 8 showed the expected 4+:0− segregations of the Snf1 phenotype; this result confirmed the gel transfer hybridization data showing that the plasmid integrated at a locus other than *SNF1* in these strains. We were surprised, however, to observe that in our initial scoring of tetrads from transformants 1, 3, 6, and 12, the Snf1 phenotype also segregated 4+:0−; segregations of 2+:0− were observed for transformant 2.

We suspected that the integrated pCN11 plasmid was excising during growth of the spore clones, thereby restoring the wild-type *SNF1*$^+$ gene. This seemed likely for three reasons. First, in each tetrad two of the germinating spores formed colonies characteristic of *snf1* mutants (small size and color typical of petite strains), and this colony morphology cosegregated with the *URA3*$^+$ marker. These data suggested that gene disruption conferred a Snf1$^-$ phenotype. Second, papillae were observed on many of these colonies; these could correspond to *SNF1*$^+$ revertants, which would grow faster than *snf1*$^-$ cells. Finally, because the spore clones were picked and grown again without selection for the *URA3* marker before scoring the Snf1 phenotype, further enrichment for *SNF1*$^+$ revertants had the opportunity to occur. A culture containing a mixture of Snf1$^-$ Ura3$^+$ and Snf1$^+$ Ura3$^-$ cells would be scored as having a Snf1$^+$ Ura3$^+$ phenotype.

To prevent possible complications caused by excision of the integrated plasmid, the *URA3*$^+$ spore clones were maintained on medium selective for uracil prototrophy before scoring the Snf1 phenotype. The Snf1 phenotype was scored by the ability to utilize raffinose. All Ura3$^+$ spores from

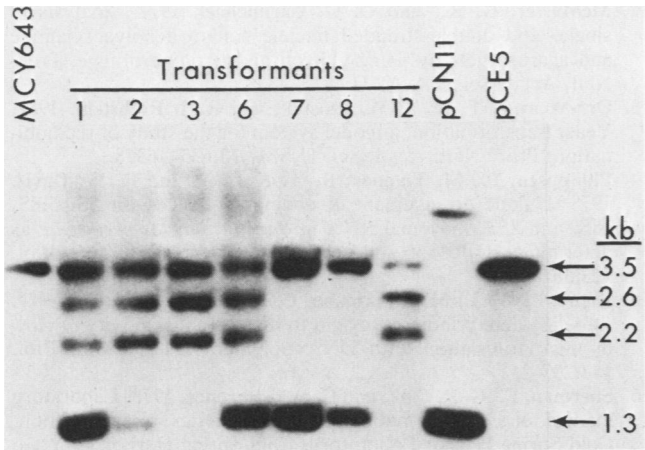

FIG. 6. Identification of transformants carrying a *SNF1* locus disrupted by plasmid integration. Genomic DNAs (3 μg) from diploid transformants 1, 2, 3, 6, 7, 8, and 12 and their parent, MCY643, were digested simultaneously with *Sal*I and *Eco*RI. Plasmid pCN11 and pCE5 DNAs (0.5 ng) were also digested with *Sal*I and *Eco*RI. The resulting fragments were separated by electrophoresis on a 1.0% agarose gel and transferred to nitrocellulose. The same 0.65-kb *Sau*3AI fragment that was subcloned in pCN11 was ^{32}P-labeled by nick translation (15) and hybridized to the filter. Homologous fragments were detected by autoradiography. The 1.3-kb fragment from pCN11 provides a marker for the size of the fragments expected from plasmid DNA integrated at the *URA3* locus or from tandem copies of the plasmid integrated at the *SNF1* locus. The 3.5-kb fragment from pCE5 (8) identifies the fragment derived from the uninterrupted genomic *SNF1* locus.

diploids 1, 2, 3, 6, and 12 now scored as Snf1$^-$. The Ura3$^+$ spores from diploids 7 and 8 again scored as Snf1$^+$.

To confirm these findings, a second set of tetrads was dissected from diploids 3, 7, and 12. As soon as these spore clones formed small colonies, they were transferred to two plates of minimal medium: one plate selective for uracil prototrophy and one plate supplemented with uracil. Cells used for testing the Snf1 phenotype were taken from the selective plate for the Ura3$^+$ spore clones and from the nonselective plate for the Ura3$^-$ clones. The Snf1 phenotype was scored by the ability to utilize sucrose, raffinose, galactose, and glycerol as the carbon source in rich medium, minimal medium selective for uracil independence, and minimal medium supplemented with uracil. By all criteria, the Snf1 phenotype was now observed to segregate 2+:2− in seven tetrads from transformants 3 and 12; moreover, all Ura3$^+$ spores were Snf1$^-$, and all Ura3$^-$ spores were Snf1$^+$. Seven tetrads from transformant 7 again showed the expected 4+:0− segregations for the Snf1 phenotype.

These data show that disruption of the cloned gene at its chromosomal locus by integration of plasmid pCN11 produces a Snf1$^-$ phenotype, thereby proving that the cloned gene is indeed the *SNF1* gene. Moreover, the phenotype of the null mutation constructed by gene disruption is indistinguishable from the Snf1$^-$ phenotype previously identified by characterization of other *snf1* alleles.

DISCUSSION

Previous genetic and biochemical evidence implicated the *SNF1* gene in regulation of gene expression by glucose repression. The *SNF1* gene product appears to act positively to derepress expression of glucose-repressible genes when cells are grown under conditions of limiting glucose. An understanding of the function of the *SNF1* gene product would provide insight into the workings of an important regulatory system in *S. cerevisiae*. These studies represent the first step in that direction.

We located the *SNF1* gene on a cloned DNA segment and identified the 2.4-kb poly(A)-containing RNA encoded by the gene. The RNA coding region was mapped, and the direction of transcription was determined. No intron was detected, although the existence of an intron very close to the 5′ or 3′ end of the transcriptional unit cannot be excluded. The level of stable *SNF1* RNA was the same in glucose-repressed and -derepressed cells. This finding suggests that expression of the *SNF1* gene is not glucose repressible; however, it seems likely that the *SNF1* gene encodes a protein, and regulation at the translational level is not yet excluded. We favor the possibility that the *SNF1* protein is constitutively synthesized and plays a role in the primary response to glucose availability. A *snf1* mutant strain was indistinguishable from the wild type with respect to their *SNF1* RNA levels; it therefore seems unlikely that the *SNF1* gene product is involved in regulating expression of its structural gene. The *SNF1* RNA is not abundant; comparison with the *URA3* RNA in RNA gel transfer hybridization experiments suggests that the amount of *SNF1* RNA in cells is approximately one-half that of *URA3* RNA (unpublished data).

To confirm the identity of the cloned *SNF1* gene, we used the cloned DNA to disrupt the gene at its chromosomal locus by applying the method of Shortle et al. (17). Gene disruption conferred a Snf1$^-$ phenotype. This experiment demonstrated that the cloned gene is *SNF1*. In addition, mutants carrying the null allele created by gene disruption displayed a Snf1$^-$ phenotype indistinguishable from that conferred by previously isolated *snf1* mutations. The observed phenotype therefore results from loss of *SNF1* gene function.

Although we recovered no Snf1$^-$ haploid transformants among the six transformants in our gene disruption experiment, Snf1$^-$ colonies would undoubtedly have been included if we had obtained a larger number of transformants.

The gene disruption experiment involved insertion of a plasmid at the *SNF1* chromosomal locus accompanied by duplication of a 0.65-kb sequence. We observed an apparently high rate of excision of the plasmid, presumably by homologous recombination of the duplicated region, under conditions nonselective for maintenance of the integrated plasmid. We were unable to determine the recombination frequency because excision restored the *SNF1* gene, which confers a selective advantage relative to the disrupted *snf1* allele.

This work provides a foundation for studies of the *SNF1* gene product. Efforts are under way to obtain antibody directed against the protein presumably encoded by the cloned *SNF1* gene. The availability of such an antibody will facilitate identification of the *SNF1* protein and analysis of its role in regulation.

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