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Cloning and Genetic Mapping of *SNF1*, a Gene Required for Expression of Glucose-Repressible Genes in *Saccharomyces cerevisiae*

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A functional *SNF1* gene product is required to derepress expression of many glucose-repressible genes in *Saccharomyces cerevisiae*. Strains carrying a *snf1* mutation are unable to grow on sucrose, galactose, maltose, melibiose, or nonfermentable carbon sources; utilization of these carbon sources is regulated by glucose repression. The inability of *snf1* mutants to utilize sucrose results from failure to derepress expression of the structural gene for invertase at the RNA level. We isolated recombinant plasmids carrying the *SNF1* gene by complementation of the *snf1* defect in *S. cerevisiae*. A 3.5-kilobase region is common to the DNA segments cloned in five different plasmids. Transformation of *S. cerevisiae* with an integrating vector carrying a segment of the cloned DNA resulted in integration of the plasmid at the *SNF1* locus. This result indicates that the cloned DNA is homologous to sequences at the *SNF1* locus. By mapping a plasmid marker linked to *SNF1* in this transformant, we showed that the *SNF1* gene is located on chromosome IV. We then mapped *snf1* to a position 5.6 centimorgans distal to *rna3* on the right arm; *snf1* is not extremely closely linked to any previously mapped mutation.

The *SNF1* gene (sucrose nonfermenting) was first identified as a gene essential for sucrose utilization. Five recessive *snf1* alleles were isolated in a search for sucrose-nonfermenting mutants of *Saccharomyces cerevisiae* S288C (3). The *snf1* mutants were found to be pleiotropically defective for growth on galactose, maltose, melibiose (unpublished data), and several nonfermentable carbon sources; utilization of all these carbon sources is regulated by glucose repression. A *snf1* mutation prevents expression of the fermentation-positive phenotypes of all members of the *SUC* and *MAL* gene families tested (*SUC2*, *SUC7*, *MAL2*, *MAL3*, and *MAL4*) (3). The *snf1* mutants also show a reduced capacity for growth on glucose which is remedied by sufficiently high glucose concentrations. Homozygous *snf1* diploids are defective in sporulation. This defect and the inability to use nonfermentable carbon sources cannot be attributed to the lack of a functional mitochondrial genome (3). These findings suggest that the *SNF1* gene product is required to derepress expression of glucose-repressed genes in response to low glucose concentrations in the growth medium.

The biochemical basis of the sucrose fermentation defect in *snf1* mutants has also been investigated (2). Strains derived from S288C carry the *SUC2* structural gene for the sucrose-hydrolyzing enzyme invertase. Wild-type cells produce two forms of invertase: a secreted, glycosylated form necessary for sucrose utilization and a cytoplasmic, nonglycosylated form. The two forms are encoded by two *SUC2* mRNAs, 1.8 and 1.9 kilobases (kb) in size, that differ at their 5′ ends (2). The 1.8-kb mRNA encodes cytoplasmic invertase, and the 1.9-kb mRNA encodes a signal peptide-containing precursor to secreted invertase (2, 4). The 1.8-kb *SUC2* mRNA and the cytoplasmic invertase are produced constitutively in both wild-type and *snf1* mutant cells. The amount of stable 1.9-kb mRNA and the synthesis of secreted invertase are regulated by glucose repression in wild-type cells. In contrast, *snf1* mutants fail to produce the 1.9-kb mRNA or secreted invertase during growth in limiting glucose (2). The *SNF1* gene product therefore appears necessary for derepression of *SUC2* gene expression at the RNA level.

These genetic and biochemical data indicate that the *SNF1* gene is involved in regulation of gene expression by glucose repression. The evidence suggests that the *SNF1* gene product acts as a positive activator to derepress expression of glucose-repressible genes in response to conditions of low external glucose concentration. Further understanding of the molecular mechanism by which the *SNF1* gene product functions requires isolation of the gene and identification of the gene product. We report here the isolation of recombinant plasmids containing the cloned *SNF1* gene. We used the cloned DNA to facilitate assignment of the gene to a chromosome and then determined the genetic map position of *snf1*, thereby showing that *snf1* is not very closely linked to any previously mapped mutation. Further studies of the structure and expression of the *SNF1* gene are reported in the accompanying paper (5).

MATERIALS AND METHODS

Strains and general genetic methods. *S. cerevisiae* strains and genotypes are listed in Table 1. Standard yeast genetic procedures of crossing, sporulation, and tetrad analysis were followed (11, 16). Sporulation was carried out on solid medium (3). For tetrad analysis, diploids were purified by single-colony isolation before sporulation. Media and methods for scoring ability to utilize carbon sources have been described previously (3); utilization of fermentable sugars was scored on rich medium (YEP) containing 2% of the sugar under anaerobic conditions.

Restriction enzymes, preparation of plasmid DNA, and gel electrophoresis of DNA. Restriction enzymes were purchased from New England BioLabs. Unless otherwise specified, plasmid DNAs were purified by cesium chloride-ethidium bromide equilibrium centrifugation. Agarose gel electropho-

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TABLE 1. *S. cerevisiae* strains

Strain	Genotype	Source or reference
MCY364[a]	*MATα his4-539 lys2-801 snf1-28 SUC2*$^+$	This work
MCY379[a]	*MATa lys2-801 SUC2*$^+$	This work
MCY417[a]	*MATa ura3-52 his4-539 snf1-31 SUC2*$^+$ *gal2*	This work
MCY419[a]	*MATα ura3-52 lys2-801 his4-539 snf1-31 SUC2*$^+$	This work
MCY420[a]	*MATα ura3-52 his4-539 snf1-31 SUC2*$^+$ *gal2*	This work
MCY421[a]	*MATα ura3-52 his4-539 SUC2*$^+$	This work
MCY553[a]	*MATa ura3-52 lys2-801 snf1-28 SUC2*$^+$	This work
MCY644	*MATa rna3 trp4 ade8 leu2 SUC2*$^+$	This work
MCYR23[a]	*MATα ura3-52 snf1-31*::pCN1 *his4-539 SUC2*$^+$ *gal2*	This work
MCYR24[a]	*MATα ura3-52 snf1-31*::pCN1 *his4-539 SUC2*$^+$ *gal2*	This work
MCYR27	*MATα ura3 snf1-31*::pCN1 *spo11 cyh2 his4-539 ade2 can1*	This work
MCYR28	*MATα ura3 snf1-31*::pCN1 *spo11 cyh2*	This work
K382-23A	*MATa spo11 ura3 can1 cyh2 ade2 his7 hom3*	(9)
K398-4D	*MATa spo11 ura3 ade6 arg4 aro7 asp5 met14 lys2 pet17 trp1*	(9)
K399-7D	*MATa spo11 ura3 his2 leu1 lys1 met4 pet8*	(9)
K396-11A	*MATa spo11 ura3 ade1 his1 leu2 lys7 met3 trp5*	(9)
F338	*MATa lys4 gal2*	G. Fink
X3271-1C	*MATa aro1D pet14 rna3 ade8 trp4 mal leu2 gal4 SUC2*$^+$	Yeast Genetic Stock Center
DBY649	*MATa his4-619 can*^{r} *cdc9*	D. Botstein

[a] Strains are isogenic or congenic to S288C.

resis was carried out in 89 mM Tris-hydrochloride–89 mM boric acid–2.5 mM EDTA (pH 8.3).

Yeast transformation and selection of plasmids complementing *snf1*. Transformation of *S. cerevisiae* was carried out by the procedure of Hinnen et al. (8). For the isolation of recombinant plasmids complementing *snf1*, plasmid DNA from each of the three clone pools in the library described previously (2) was used to transform *S. cerevisiae* to uracil prototrophy. Transformants were recovered from the regeneration agar as before (2), and transformants carrying a plasmid able to complement *snf1* were selected by spreading cells on YEP-sucrose medium and incubating anaerobically (3). Twenty-one sucrose-fermenting transformants were purified by single-colony isolation, and total cellular DNA was prepared from each transformant by the method of Davis et al. (6). Plasmids were isolated by using these DNAs to transform *Escherichia coli* HB101 to ampicillin resistance by the calcium chloride procedure (10). Plasmid DNAs were prepared from the bacteria for restriction site analysis by a modification of the alkaline lysis method (10).

Subclone construction. To subclone the *Bam*HI fragment from plasmid pCE1, plasmid DNA was digested with *Bam*HI and *Sal*I. YIp5 DNA (1) was digested with *Bam*HI and treated with calf intestinal phosphatase (Boehringer Mannheim) to prevent later self-ligation. Both DNAs were extracted with phenol and precipitated with ethanol. The DNAs were then mixed and ligated with T4 DNA ligase (a gift of J. van Oostrum) in 50 mM Tris-hydrochloride (pH 7.8)–10 mM $MgCl_2$–20 mM dithiothreitol–0.5 mM ATP at 14°C overnight. The ligated DNA was used to transform *E. coli* HB101 to ampicillin resistance, and plasmid DNAs were prepared as described above. Plasmid pCN1 was recovered (Fig. 1).

Assay for invertase. Glucose-repressed cells were prepared by growing cells in rich medium (YEP) containing 2% glucose at 30°C with aeration. Cells growing exponentially (50 Klett units, measured with a Klett-Summerson photoelectric colorimeter with a green filter) were collected by centrifugation and chilled. Derepressed cells were obtained by shifting glucose-repressed cells into medium containing 0.05% glucose for further growth: glucose-repressed cells prepared as above were washed twice, suspended in YEP–0.05% glucose, and grown with aeration for 2 h at 30°C. Cells were harvested by centrifugation and chilled. Before invertase was assayed, glucose-repressed and -derepressed cells were washed twice with cold 10 mM sodium azide. Secreted invertase (located in the periplasmic space) was then assayed essentially as described by Goldstein and Lampen (7), except that the reaction was carried out at pH 5.1 and 37°C.

Chromosome localization. The method of Klapholz and Esposito (9) was followed for chromosome localization. Strains MCYR27 and MCYR28 were recovered as spore clones from the MCYR24 × K382-23A cross. These strains were shown to carry the *spo11* mutation by complementation testing (9).

Genetic mapping. Linkage data were based on tetrads with four viable spores. For analysis of the MCY364 × MCY644 cross, the *snf1* marker was scored by assaying growth on sucrose and glycerol, and the *rna3* marker was scored by assaying growth at 25°C (permissive temperature) and 36°C (restrictive temperature).

RESULTS

Isolation of recombinant plasmids complementing the *snf1* mutation. Recombinant plasmids containing the *SNF1* gene were recovered from a plasmid library by selecting for their ability to complement a *snf1* mutation in *S. cerevisiae*. The library contains recombinant plasmids representing the genome of a strain derived from the S288C strain of *S. cerevisiae*. Construction of the library has been described previously (2). Total genomic DNA was partially digested with *Sau*3AI, and large fragments were purified and inserted in the *Bam*HI site of the episomal vector YEp24. This vector includes pBR322 DNA, the *S. cerevisiae URA3* gene, and a fragment of the 2-μm circle; it replicates and can be selected in *E. coli* and *S. cerevisiae* (1). DNA from this library was used to transform two *ura3 snf1 SUC2*$^+$ yeast strains, MCY417 and MCY419, to uracil independence. Those Ura3$^+$ transformants that were concomitantly transformed to a Snf1$^+$ phenotype were then isolated by selection for growth on sucrose as described above. A *snf1* mutant is unable to utilize sucrose because a functional *SNF1* gene product is required for derepression of the *SUC2* mRNA encoding secreted invertase (2).

Twenty-one sucrose-fermenting transformants were chosen for further analysis. Of these, six were tested to confirm that the Snf1$^+$ phenotype was conferred by a recombinant plasmid rather than by a chromosomal reversion event. The six transformants were grown without selection for uracil prototrophy to allow segregation of the plasmid; uracil-requiring segregants were recovered and tested for concomitant loss of the Snf1$^+$ phenotype. All six transformants

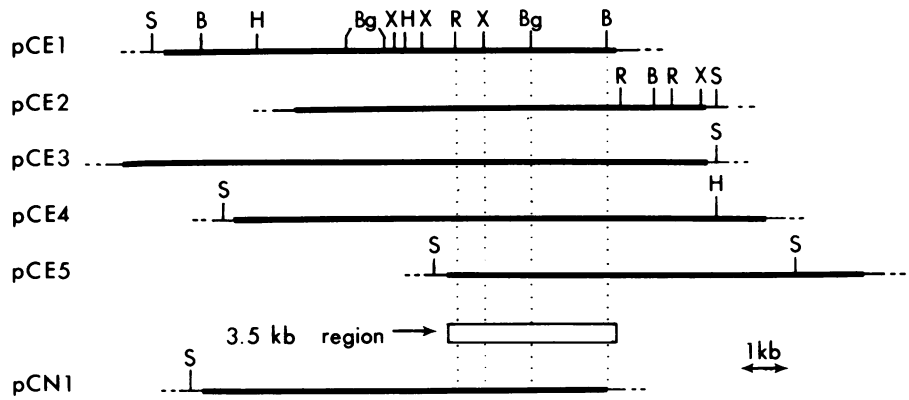

FIG. 1. Restriction maps of recombinant plasmids complementing the *snf1* mutation. The solid bars represent the cloned yeast DNA. The thin lines represent the plasmid vector DNA. The *Sal*I site in the vector is shown to indicate the orientation of the cloned yeast DNA with respect to the vector DNA. The open bar indicates the 3.5-kb region common to pCE1 through pCE5. Plasmid pCN1 is a subclone derived from pCE1 (see text). Restriction sites: B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; R, *Eco*RI; S, *Sal*I; X, *Xho*I.

showed cosegregation of the Ura3$^+$ and Snf1$^+$ phenotypes, indicating that in each case the plasmid was required for complementation of the *snf1* defect.

To determine whether the same cloned gene was responsible for the complementation in all Snf1$^+$ transformants, we recovered and characterized plasmid DNAs from the twenty-one transformants. Total cellular DNA was prepared from each strain and was used to transform *E. coli* to ampicillin resistance. Plasmid DNAs were prepared from the *E. coli* transformants for restriction site analysis. Gel electrophoresis of the DNAs digested with *Hin*dIII, *Eco*RI, *Bam*HI, or *Xho*I revealed that the inserted yeast DNA in 19 plasmids gave rise to common fragments. The two remaining plasmids did not complement a *snf1* mutation upon subsequent transformation of *S. cerevisiae*. Five different plasmids, pCE1 through pCE5, were represented among the 19 plasmids. The restriction maps of these five plasmids indicate that they contain overlapping DNA segments derived from the same chromosomal locus (Fig. 1). All the plasmids include a common 3.5-kb region.

To confirm that this cloned DNA complements the *snf1* defect, DNA from plasmid pCE1 was used to transform MCY417 to uracil prototrophy. All the Ura$^+$ transformants showed a Snf1$^+$ phenotype, as assayed by their ability to utilize sucrose and glycerol as carbon sources, and all mitotic Ura3$^-$ segregants derived from these transformants were Snf1$^-$.

Thus far, complementation was scored on the basis of carbon source utilization. Next, an experiment was carried out to assess the ability of the cloned DNA to complement *snf1* with respect to regulation of *SUC2* gene expression. In a *SNF1*$^+$ background, expression of the *SUC2* gene is regulated by glucose repression; the *SUC2* mRNA encoding secreted invertase is absent from glucose-repressed cells and is present in derepressed cells (2). Because the cloned DNA complemented the *snf1* defect in sucrose fermentation, it seemed likely that strains transformed with the cloned DNA produced the secreted form of invertase under derepressing conditions; however, it was not evident that the cloned DNA restored the normal regulation observed in a *SNF1*$^+$ strain. We therefore assayed secreted invertase in strain MCY417 transformed with pCE1 after growth under glucose-repressing and -derepressing conditions as described above. The transformant produced wild-type levels of secreted invertase with normal regulation by glucose repression (Table 2). These data indicate that the cloned DNA complements the *snf1* mutation to give a wild-type phenotype with respect to regulation of *SUC2* gene expression. It is worth noting that the transformant probably carries multiple copies of pCE1 because pCE1 is derived from the multicopy plasmid vector YEp24.

We concluded from these experiments that the 3.5-kb region common to the five plasmids complements the *snf1* mutation in *S. cerevisiae*. Because no other cloned DNA segment capable of complementing *snf1* was recovered, it seemed likely that this 3.5-kb region contained the *SNF1* gene.

Integration of cloned DNA at the *SNF1* locus. In *S. cerevisiae*, integration of exogenously added DNA into the chromosomes occurs by homologous recombination (8). We therefore expected that if the cloned 3.5-kb region contained the *SNF1* gene, this DNA would integrate by recombination with the homologous sequence at the *SNF1* chromosomal locus. To test this prediction, we subcloned the *Bam*HI fragment from pCE1 that contains most of the 3.5-kb region (Fig. 1) into the integrating vector YIp5 as described above. Plasmid pCN1 was recovered (Fig. 1). It carries the *URA3* gene of YIp5. Plasmid pCN1 DNA was used to transform MCY420 (*ura3 snf1*) to uracil independence. Stable Ura$^+$ transformants could result from integration of the plasmid either at the *URA3* locus or at a site homologous to the subcloned *Bam*HI fragment. Orr-Weaver et al. reported that linearization of a plasmid by cleavage within the cloned yeast DNA before transformation increases the recovery of integrants and targets the integration to chromosomal sites homologous to the cleaved sequence (14). They also showed

TABLE 2. Regulation of secreted invertase in transformed strains

Strain	Relevant genotype	Invertase activity[a]	
		Derepressed	Repressed
MCY420	*snf1-31 SUC2*$^+$	<0.05	<0.05
MCY421	*SNF1 SUC2*$^+$	0.35	<0.05
MCY417(pCE1)	*snf1-31 SUC2*$^+$ (pCE1)	0.35	<0.05
MCYR24	*snf1-31*::pCN1 *SUC2*$^+$	0.30	<0.05

[a] Micromoles of glucose released per minute per milliliter of cells at 50 Klett units.

TABLE 3. Ura3 and Trp1 phenotypes of spore clones

Cross	No. of clones of phenotype:			
	Ura3$^+$ Trp1$^+$	Ura3$^-$ Trp1$^-$	Ura3$^+$ Trp1$^-$	Ura3$^-$ Trp1$^+$
MCYR27 × K398-4D	33	62	0	2
MCYR28 × K398-4D	37	64	0	0

that a gap in the cloned DNA is as effective as a single break. We targeted integration of plasmid pCN1 by digesting plasmid DNA with *Xho*I, thereby producing a gap within the *SNF1*-complementing region (Fig. 1). Two independent, stable Ura$^+$ transformants, MCYR23 and MCYR24, were chosen for study. Both were transformed to a Snf1$^+$ phenotype, as judged by their ability to utilize sucrose and glycerol. MCYR24 was also shown to synthesize secreted invertase (Table 2). To determine whether plasmid integration occurred at the *snf1* locus, each transformant was crossed to the *SNF1*$^+$ *URA3*$^+$ strain MCY379, and the resulting diploids were sporulated and subjected to tetrad analysis. If integration occurred at the *SNF1* locus, both homologs in the diploid would carry a *SNF1*$^+$ allele at the *SNF1* locus, and all tetrads would show 4+:0− segregations for the Snf1 phenotype. If integration occurred at a locus unlinked to *SNF1*, 4+:0−, 3+:1−, and 2+:2− segregations would be observed. The Snf1 phenotype segregated 4+:0− in all tetrads (9 tetrads from one cross and 11 from the other). This finding indicates that in both transformants, pCN1 integrated at a site linked to *SNF1*. The *URA3* gene of pCN1 should therefore be linked to *SNF1* on one homolog in the diploid and unlinked to the chromosomal *URA3* locus. The segregation of *URA3* was consistent with this conclusion. The Ura3 phenotype segregated 4+:0−, 3+:1−, and 2+:2−, as would be predicted for a diploid heterozygous (*URA3*$^+$/*ura3*) at the *URA3* locus on chromosome V and also carrying an unlinked *URA3*$^+$ gene. Other markers in both crosses segregated 2:2.

For transformant MCYR24, we carried out an additional experiment to confirm that the plasmid had integrated at only one chromosomal location. MCYR24, which was derived from a *snf1 ura3* strain by transformation, was crossed to the *snf1 ura3* strain MCY553. Tetrad analysis of the diploid showed 2+:2− segregations for the Snf1 and Ura3 phenotypes and cosegregation of *SNF1* and *URA3* in the seven tetrads examined. These findings indicate that the plasmid probably integrated at only one locus, but they do not exclude the possibility that integration occurred at two or more tightly linked loci. In either case, because the *SNF1* marker segregated 2+:2− in this cross, we can rule out the possibility that the 4+:0− segregations observed above resulted from segregation of multiple, unlinked *SNF1*$^+$ genes. The conclusion that integration of pCN1 occurred at a site linked to the chromosomal *SNF1* locus is valid.

These experiments show that the cloned DNA that complements the *snf1* mutation is homologous to sequences linked to the chromosomal *SNF1* locus. This finding is consistent with, but does not prove, the idea that the cloned region contains the *SNF1* gene.

***SNF1* gene is located on chromosome IV.** Our analysis of transformant MCYR24 showed that integration of plasmid pCN1 resulted in linkage of the *URA3* marker to the *SNF1* locus. We used this *URA3* marker to facilitate identification of the chromosome carrying *SNF1* by the method of Klapholz and Esposito (9). This method relies on the fact that diploids homozygous for the *spo11* mutation undergo meiosis without crossing over. As a result, two markers on the same chromosome appear tightly linked, regardless of their map distance, and two markers on different chromosomes segregate independently.

We first constructed two strains carrying the *spo11* mutation, the cycloheximide resistance marker *cyh2*, and the *URA3* marker linked to *SNF1* (MCYR27 and MCYR28) as described above. Each was then crossed to three *ura3 spo11 CYH2*$^+$ strains (K398-4D, K399-7D, K396-11A), each containing markers representing a subset of the yeast chromosomes. The diploids were cycloheximide sensitive. Analysis of cycloheximide-resistant spore clones derived from the six diploids showed that *URA3* segregated independently of all markers tested except *trp1*, the chromosome IV marker from K398-4D. *URA3*$^+$ appeared linked to *TRP1*$^+$ (Table 3). These data indicate that the *SNF1* locus is on the same chromosome as *trp1*, chromosome IV.

Genetic map position of *SNF1*. We next determined the map position of *SNF1* on chromosome IV by standard tetrad analysis. First, a strain carrying the *snf1* mutation, MCY419, was crossed to several strains marked on chromosome IV: F338 (*lys4*), X3271-1C (*rna3 aro1D pet14 ade8 trp4*), and DBY649 (*cdc9*). Seven tetrads from each of the three crosses were examined for segregation of the chromosome IV markers and *snf1*, which was scored by its sucrose-nonfermenting phenotype. Linkage was observed between *snf1* and *rna3* in the cross to X3271-1C; five of the seven tetrads were parental ditype and two were tetratype.

To map the *snf1* mutation more precisely, extensive tetrad analysis was carried out on the cross MCY364 (*snf1 SUC2*$^+$) × MCY644 (*rna3 trp4 ade8 SUC2*$^+$). MCY644 was derived from the cross MCY419 × X3271-1C. Table 4 shows the linkage data. The map distance between *snf1* and *rna3* was 5.6 centimorgans (cM); between *snf1* and *ade8*, 38.2 cM; and between *ade8* and *rna3*, 34.4 cM. To determine the gene order, we examined the 11 tetrads that were tetratype for the *snf1-rna3* marker pair. Three tetrads were disregarded because of multiple crossing over or gene conversion of *ade8*. Seven tetrads were parental ditype for the *rna3-ade8* marker pair and tetratype for the *snf1-ade8* pair. Only one tetrad had the opposite configuration (tetratype for the *rna3-ade8* pair and parental ditype for the *snf1-ade8* pair) and thus were probably the result of multiple crossing over. These data indicate that the gene order from the centromere outward is *ade8 rna3 snf1*. Thus, *snf1* is the most centromere-distal marker on the right arm of chromosome IV. It is not extremely closely linked to any previously mapped gene (13).

TABLE 4. Genetic mapping of *snf1*[a]

Gene pair	PD	NPD	T	Map distance (cM)[b]
snf1-rna3	87	0	11	5.6
snf1-ade8	32	2	59	38.2
ade8-rna3	39	2	52	34.4[c]
ade8-trp4	37	0	56	30.1[d]

[a] Abbreviations: PD, parental ditype; NPD, nonparental ditype; T, tetratype.

[b] Genetic map distances in cM were calculated by the equation described by Perkins (15): cM = [(T + 6NPD)/2(PD + NPD + T)] × 100.

[c] Previously reported distance was 41.0 cM (12).

[d] Previously reported distance was 36.9 cM (12).

DISCUSSION

We isolated from our library five different recombinant plasmids that complement the *snf1* mutation in *S. cerevisiae*. Restriction site analysis suggests that these plasmids carry overlapping yeast DNA segments derived from the same chromosomal locus. A 3.5-kb region is common to all five and presumably contains the complementing gene. The cloned region complements a *snf1* mutation with respect to all defects tested: utilization of sucrose, galactose, and glycerol and regulation of synthesis of secreted invertase. We recovered no other cloned sequence able to complement *snf1*; each of the 19 complementing plasmids examined was identical to one of the five prototypic plasmids. These data suggest that the 3.5-kb region contains the *SNF1* gene.

Another line of evidence also indicates that the cloned DNA was derived from the *SNF1* locus. A restriction fragment including the 3.5-kb region was subcloned into the integrating plasmid vector YIp5 to construct plasmid pCN1. Transformation of *S. cerevisiae* with pCN1 resulted in integration of the plasmid at a chromosomal site genetically linked to the *SNF1* locus. Because integration occurs by homologous recombination in *S. cerevisiae*, this experiment indicates that the cloned DNA is homologous to DNA sequences at or near the *SNF1* locus.

These data strongly suggest, but do not prove, that the cloned DNA contains the *SNF1* gene. In the accompanying paper (5), we report further characterization of the structure and expression of the putative *SNF1* gene, and we demonstrate conclusively that the cloned gene is *SNF1*.

We also mapped the *snf1* mutation to a position 5.6 cM distal to *rna3* on chromosome IV; *snf1* is the most centromere-distal marker on the right arm of chromosome IV. We have ruled out many possibilities for the identity of the *SNF1* gene by showing that it is not extremely closely linked to any previously mapped gene.

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LITERATURE CITED

1. **Botstein, D., S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis.** 1979. Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene **8**:17–24.
2. **Carlson, M., and D. Botstein.** 1982. Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28**:145–154.
3. **Carlson, M., B. C. Osmond, and D. Botstein.** 1981. Mutants of yeast defective in sucrose utilization. Genetics **98**:25–40.
4. **Carlson, M., R. Taussig, S. Kustu, and D. Botstein.** 1983. The secreted form of invertase in *Saccharomyces cerevisiae* is synthesized from mRNA encoding a signal sequence. Mol. Cell. Biol. **3**:439–447.
5. **Celenza, J. L., and M. Carlson.** 1984. Structure and expression of the *SNF1* gene of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:54–60.
6. **Davis, R. W., M. Thomas, J. Cameron, T. P. St. John, S. Scherer, and R. A. Padgett.** 1980. Rapid DNA isolations for enzymatic and hybridization analysis. Methods Enzymol. **65**:404–411.
7. **Goldstein, A., and J. O. Lampen.** 1975. β-D-Fructofuranoside fructohydrolase from yeast. Methods Enzymol. **42C**:504–511.
8. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. U.S.A. **75**:1929–1933.
9. **Klapholz, S., and R. Esposito.** 1982. A new mapping method employing a meiotic rec⁻ mutant of yeast. Genetics **100**:387–412.
10. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **Mortimer, R. K., and D. C. Hawthorne.** 1969. Yeast genetics, p. 385–460. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts, vol. 1. Academic Press, Inc., New York.
12. **Mortimer, R. K., and D. Schild.** 1980. Genetic map of *Saccharomyces cerevisiae*. Microbiol. Rev. **44**:519–571.
13. **Mortimer, R. K., and D. Schild.** 1981. Appendix II. Genetic map of *Saccharomyces cerevisiae*, p. 641–651. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*, life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Orr-Weaver, T. L., J. W. Szostak, and R. J. Rothstein.** 1981. Yeast transformation: a model system for the study of recombination. Proc. Natl. Acad. Sci. U.S.A. **78**:6354–6358.
15. **Perkins, D. D.** 1949. Biochemical mutants in the smut fungus *Ustilago maydis*. Genetics **34**:607–626.
16. **Sherman, F., G. R. Fink, and C. W. Lawrence.** 1978. Laboratory manual for a course, methods in yeast genetics, revised edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.