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Suc1$^+$ Encodes a Predicted 13-Kilodalton Protein That Is Essential for Cell Viability and Is Directly Involved in the Division Cycle of *Schizosaccharomyces pombe*

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***Suc1*$^+$ was originally identified as a DNA sequence that, at high copy number, rescued *Schizosaccharomyces pombe* strains carrying certain temperature-sensitive alleles of the *cdc2* cell cycle control gene. We determined the nucleotide sequence of a 1,083-base-pair *Suc1*$^+$ DNA fragment and S1 mapped its 866-nucleotide RNA transcript. The protein-coding sequence of the gene is interrupted by two intervening sequences of 115 and 51 base pairs. The predicted translational product of the gene is a protein of 13 kilodaltons. A chromosomal gene disruption of *Suc1*$^+$ was constructed in a diploid *S. pombe* strain. Germinating spores carrying a null allele of the gene were capable of very limited cell division, following which many cells became highly elongated. The *Suc1*$^+$ gene was also strongly overexpressed under the control of a heterologous *S. pombe* promoter. Overexpression of *Suc1*$^+$ is not lethal but causes a division delay such that cells are approximately twice the normal length at division. These data suggest that *Suc1*$^+$ encodes a protein which plays a direct role in the cell division cycle of *S. pombe*.**

Among the many genes required for completion of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*, the *cdc2*$^+$ gene is of particular interest because it plays a role in both the G_1 and the G_2 phases of the cell cycle. The activity of the gene is required in G_1 before DNA replication and in G_2 before initiation of mitosis (24). There is also physiological and genetic evidence that *cdc2*$^+$ is not only required in G_1 and G_2 but acts at the rate-limiting step which controls the rate of progression into the S phase and nuclear division (25).

The gene product of *cdc2*$^+$ shares 62% sequence homology with the product of the *CDC28* cell cycle "start" gene of the distantly related budding yeast *Saccharomyces cerevisiae* (15, 18). Furthermore, the *CDC28* gene can rescue *cdc2*(Ts) mutants of *S. pombe* (1), and the *cdc2*$^+$ gene can rescue *cdc28*(Ts) strains of *S. cerevisiae* (7). Both genes encode products which have protein kinase activity (26, 30).

The *cdc2*$^+$ gene was physically isolated from a gene bank of *S. pombe* DNA by transformation and rescue of a temperature-sensitive *cdc2-33 S. pombe* strain (1). During screening for DNA sequences which restored a cdc$^+$ phenotype to a *cdc2-33* strain, not only *cdc2*$^+$ but also a second, previously unidentified gene known as *Suc1*$^+$ was isolated (14). The *Suc1*$^+$ gene, carried on a multicopy number vector pDB248 (2), allowed a *cdc2-33* strain to propagate at a nonpermissive temperature. This effect was found to be specific for certain alleles of *cdc2*. Of the temperature-sensitive alleles tested, *cdc2-33*, *cdc2-56*, and *cdc2-L7* were rescued by *Suc1*$^+$, whereas *cdc2-M35*, *cdc2-M63*, *cdc2-M26*, and *cdc2-M55* were not rescued (14).

It has been suggested that the *Suc1*$^+$ gene product interacts with the product of *cdc2*$^+$, but there is presently no evidence that *Suc1*$^+$ plays a direct role in the cell division cycle. In the present study, we determined the nucleotide sequence of *Suc1*$^+$, identified its protein coding sequence, and constructed strains that carry either a null allele of *Suc1*$^+$ or strongly overexpress the gene. The data suggest that the *Suc1*$^+$ gene product may indeed be involved in the cell division cycle.

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MATERIALS AND METHODS

Yeast strains, vectors, and transformation. *S. pombe* strains were cultured either in rich medium YEA (0.5% yeast extract, 3% glucose, 75 μg of adenine per ml) or in minimal medium (22) buffered with 50 mM phthalate, pH 5.6. Amino acids were supplemented at 75 μg/ml as required.

S. pombe SP36 *h*$^{-S}$ *leu1-32 cdc2-33* was used for assay of *Suc1* activity. The strain was transformed (2, 3) to Leu$^+$ at 25°C with plasmids carrying the *Suc1* gene. The transformants were transferred from 25 to 36°C and, if cell division continued, the transforming plasmid was defined as *Suc1*$^+$.

The replicating *S. pombe* vectors used were pDB248 (2) and pIRT2. pIRT2 is based on pUC118, itself a derivative of pUC18 (23), which carries the intergenic region of bacteriophage M13 (J. Vierira, personal communication). pUC118 was converted to pIRT2 by insertion of a 1.2-kilobase (kb) *Eco*RI restriction fragment of *S. pombe* DNA carrying *ars1* (19) into the unique *Eco*RI site of pUC118, followed by insertion of a 2.2-kb *Sal*I-*Xho*I restriction fragment carrying the *LEU2* gene of *S. cerevisiae* (5) into the unique *Hin*dIII site of the polylinker. The resulting vector, pIRT2, has unique *Sph*I, *Pst*I, *Sal*I, *Bam*HI, *Sma*I, and *Sst*I restriction sites. *Suc1* DNA fragments (see Fig. 1) were inserted into these sites.

The *Suc1*::*leu* allele was constructed for gene replacement as follows. A 2.7-kb *Hin*dIII genomic restriction fragment carrying *Suc1*$^+$ (see below) was inserted into the unique *Hin*dIII site of the bacterial vector pTR262 (27). A 2.2-kb *Sal*I-*Xho*I restriction fragment carrying the *LEU2* gene of *S. cerevisiae* was blunt ended with the Klenow fragment of DNA polymerase and ligated with the unique *Xba*I site within *Suc1* which had been similarly blunt ended. *Hin*dIII digestion of this construction, p*Suc1*::*leu*, separates the *S. pombe Suc1*::*leu* fragment from the bacterial vector.

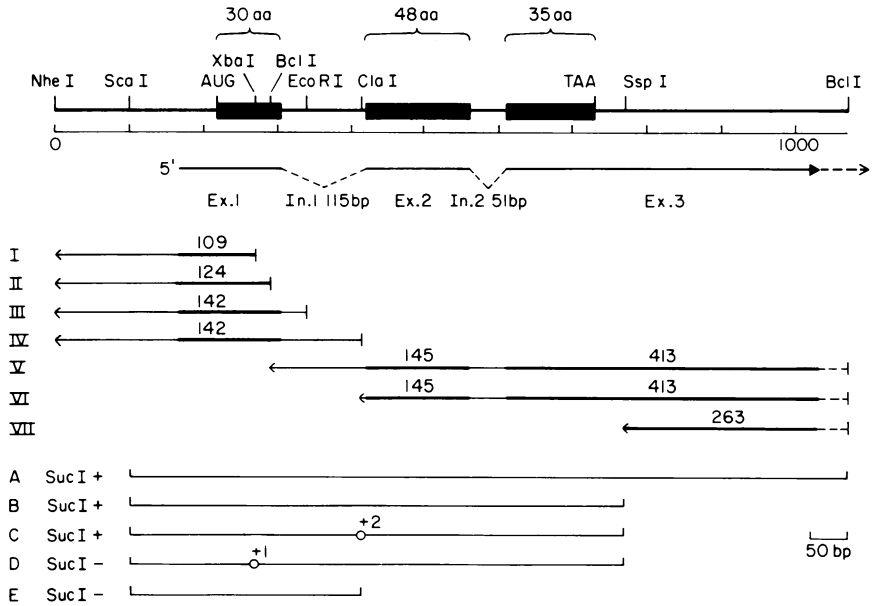

FIG. 1. Restriction map and mRNA transcript of *Suc1*$^+$. The map covers the 1,083-bp *Nhe*I-*Bcl*I restriction fragment. AUG and TAA mark the initiating and terminating sites, respectively, of the predicted *Suc1* polypeptide. The heavy lines indicate the three protein-coding regions in exon 1 (30 amino acids [aa]), exon 2 (48 amino acids), and exon 3 (35 amino acids). The pre-mRNA transcript is shown with the positions of two intervening sequences of 115 and 51 bp. The single-stranded DNA probes used for S1 mapping are indicated as I to VII. The precise limits of each are given in Materials and Methods. The heavy lines within each probe correspond to the S1-resistant fragments in Fig. 4. A to E mark a series of subclones of *Suc1* carried in pIRT2. A extends from *Sca*I to *Bcl*I, B extends from *Sca*I to *Ssp*I, C extends from *Sca*I to *Ssp*I containing a *Cla*I fill-in mutation (see the text), D extends from *Sca*I to *Ssp*I carrying an *Xba*I fill-in mutation, and E extends from *Sca*I to *Cla*I. Each construction is classified as *Suc1*$^+$ or *Suc1*$^-$ on the basis of its ability to rescue a *cdc2-33 S. pombe* strain.

Gene replacement (28) of *Suc1*$^+$ by *Suc1::leu* was achieved by transformation of diploid strain SP588 h^{+N} *ade6-216 leu1-32*/h^{-S} *ade6-210 leu1-32* to Leu$^+$ with 1 μg of *Hin*dIII-digested p*Suc1::leu*. The strain is phenotypically Ade$^+$ since the *ade6-210* and *ade6-216* alleles complement in *trans* (13). The Leu$^+$ transformants were characterized as described below. A *Suc1*$^+$/*Suc1::leu* strain derived from the parental diploid was allowed to sporulate in liquid minimal medium (22) containing 5 rather than 100 mM NH_4Cl. A preparation of pure spores, uncontaminated by unsporulated cells, was obtained by the method described previously (4).

The *Suc1*$^+$ gene was strongly overexpressed on plasmid pART1, which was derived from pIRT2 by insertion of a 700-base-pair (bp) fragment of DNA carrying the promoter of the alcohol dehydrogenase gene of (ADH) *S. pombe*. An *Sca*I-*Ssp*I fragment of *Suc1*$^+$ (see Fig. 1) was inserted into the unique *Sma*I site of pART1 such that transcription of *Suc1*$^+$ was driven by the *adh* promoter. The resulting plasmid was introduced into SP202 h^{+N} *leu1-32 ade6-216*, and stable Leu$^+$ transformants were detected after several rounds of replica plating on nonselective medium.

DNA sequencing. The presented 1,083-bp sequence of *Suc1* is part of a larger region of 2,009 bp which was initially sequenced. The sequence data were obtained from a series of overlapping clones on both DNA strands. The dideoxynucleotide method (29) was used throughout.

Transcript mapping. RNA was prepared from *S. pombe* 972 (h^{-S}) cultured at 30°C in minimal medium. Cells were harvested during exponential growth, chilled on ice, and washed in cold water. Sterile glass beads (10 ml; 0.45-mm diameter) were added to the pelleted cells, and cold breaking buffer (0.32 M sucrose, 20 mM Tris hydrochloride [pH 7.5], 10 mM EDTA, 0.5 mg of heparin per ml) was added until the meniscus just covered the glass beads. The cells were broken by vortexing for 1 min. Dilution buffer (50 mM Tris hydrochloride [pH 7.5], 100 mM NaCl, 5 mM EDTA, 1% sodium dodecyl sulfate, 0.5 mg of heparin per ml) (20 ml) was added to the broken cells with an equal volume of phenol. The mixture was vortexed vigorously, and the aqueous phase was isolated. After two further phenol extractions and one phenol-chloroform extraction, the RNA was precipitated by addition of LiCl to 0.5 M and ethanol to 70%. Northern blotting of whole-cell RNA was done exactly as previously described (20).

For S1 mapping, single-stranded DNA probes were prepared by insertion of appropriate restriction fragments of *Suc1* into pUC118, a vector which can be obtained in single-stranded form after superinfection of plasmid-bearing *Escherichia coli* (TG1) with the defective phage M13K07 (Vierira, personal communication).

Probes I, II, III, and IV were obtained as follows. The 2.7-kb *Hin*dIII fragment containing *Suc1* was inserted into the unique *Hin*dIII site in the polylinker of pUC118. Deletions of part of the *Suc1* insert were obtained by digestion with *Xba*I (probe I) *Bcl*I and *Bam*HI (probe II), *Eco*RI (probe III), and *Cla*I (filled in with Klenow) and *Sma*I (probe IV), followed in each case by self-ligation. Probes V, VI, and VII were prepared from a single construction in which the central *Bcl*I fragment of *Suc1* was inserted in the *Bam*HI site of pUC118 in the approximate orientation (see Fig. 1). Each single-stranded plasmid (1 μg) was annealed with 5 pmol of the oligonucleotide 5'GTTTTCCCAGTCACGAC (New England BioLabs no. 1212) and primer extended with ^{32}P-labeled deoxynucleotide triphosphates as described previously (10). The double-stranded DNA thus formed was digested with *Nhe*I (probes I, II, III, and IV), *Sma*I (probe V), *Cla*I (probe VI), or *Ssp*I (probe VII) (see Fig. 1). After denaturation, the single-stranded DNA probes were isolated

on 4% polyacrylamide–urea gels (21) and recovered by electroelution into dialysis bags.

The probes were annealed with 20 μg of whole-cell RNA in 0.25 M NaCl–30 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 7.6)–3 mM EDTA at 65°C for 18 h. The hybrids were digested with 100 U of S1 nuclease in 4 mM $ZnSO_4$–30 mM NaOAc (pH 4.6)–0.25 M NaCl at 37°C for 45 min. The resulting DNA fragments were resolved on either 4 or 6% polyacrylamide–urea gels.

RESULTS

Nucleotide sequence and transcript map of *Suc1*$^+$. The *Suc1*$^+$ gene (14) was originally isolated from a gene bank which had been constructed by ligation of partial *Sau3A* restriction fragments of wild-type *S. pombe* DNA into the *Bam*HI site of the *S. cerevisiae* bacterial shuttle vector pDB248 (2). Subsequently, a 14-kb *Bam*HI genomic fragment carrying *Suc1*$^+$ was isolated from a bacteriophage λ library by plaque hybridization. From this 14-kb restriction fragment, a biologically active 2.7-kb *Hin*dIII subclone was obtained and used as the starting material for the present studies (*Suc1*$^+$ activity is defined as the ability of the gene carried on either multicopy vector pDB248 or pIRT2 to rescue an *S. pombe* strain carrying the *cdc2-33* allele at the nonpermissive temperature).

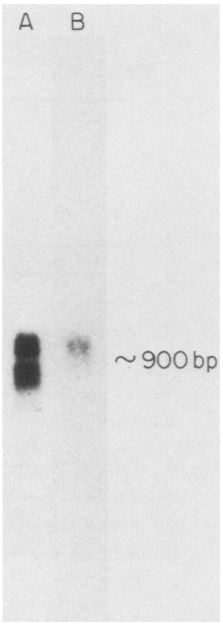

FIG. 3. Northern blot of the *Suc1*$^+$ transcript. Lane A was probed with the 799-bp *BclI-BclI* restriction fragment containing exons 2 and 3. Lane B was probed with the directly adjacent *BclI-BclI* restriction fragment of approximately 850 bp. This fragment lies immediately to the right of the restriction map in Fig. 1.

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GCTAGCGCAAAAAACCTGTTTTCTGCAACCGATAACTTTCGTTTTAGGTATAGAGTGTTG      60
Nhe I
AGTTGAAGCCCAAAGTTGTGTGTTACCTAAAGTGAGTACTGTTACTTTAAGTCTAAAACG     120
                                  Sca I
AAATTTAGCGACCTTTAACGGATCACCCAAGTCCTAGAGTATCCTATACTTTACCCATCT     180

                                                MetSerLysSerGlyValPro
CAAAGAAAAGTTATTCTTTTTGAGTATTAAGGAGGAGATATGTCGAAAAGTGGTGTGCCA     240

ArgLeuLeuThrAlaSerGluArgGluArgLeuGluProPheIleAspGlnIleHisTyr
CGTCTTTTAACTGCTTCTGAGCGTGAACGTCTAGAGCCATTCATTGATCAAATCCATTAT     300
          Xba I                                 Bcl I
SerProArg----------
TCTCCTAGGTATGATGATATTGTTAAAGAAACTGCCATACTTTGGGAATTCTCTAGGTTC     360
                                              EcoRI
------------------------------------------------------------
TTTGTTTTCCCCTAAAACGAGACTCTTGTATCTTATTTGAGCAAATGCTAATCGATGAAA     420
                                                  Cla I
----TyrAlaAspAspGluTyrGluTyrArgHisValMetLeuProLysAlaMetLeuLys
TAGATATGCTGATGATGAATATGAATATCGACATGTTATGTTACCAAAAGCAATGCTAAA     480

 AlaIleProThrAspTyrPheAsnProGluThrGlyThrLeuArgIleLeuGlnGluGlu
AGCGATACCCACCGATTATTTCAATCCAGAGACTGGAACGCTTCGAATCCTTCAAGAAGA     540

 GluTrpArgGlyLeuGlyIleThrGln----------
AGAATGGAGAGGACTTGGAATTACTCAAGTAAGTAATCAAGGATTTTAACTGCTCAAAAA     600

--------------------SerLeuGlyTrpGluMetTyrGluValHisValProGluPro
CAAACTGACATTTATTTAGAGTCTGGGATGGGAAATGTATGAAGTCCATGTCCCAGAGCC     660

 HisIleLeuLeuPheLysArgGluLysAspTyrGlnMetLysPheSerGlnGlnArgGly
ACACATCCTGCTATTTAAGCGTGAAAAAGATTATCAAATGAAATTTAGTCAACAACGGGG     720

 Glyoo
TGGTTAAGTTTGTCAACTCCAACGTTTCTGTTTTACTTTATCAAAAAAATATTAGTTCAT     780
                                                  Ssp I
GGATGCGAAACATATTGACTTTTTAGGCATTCAATACGCTGCTTTTTTTTATCAAGCTCA     840

CTGAGTGTTTCATGTGGGAGTCGCTAAAAATGATAATGAATTATCTTCGCCATTTATGTT     900

TGCTACAGACTAACAACATATTTGGGCAATTCCTACTTTTTTTTCACTGTAGATATACTT     960

TACTGGTTCTTTTTATTTTCTTTCAAAATCATCTTTCTATTTTCTTTTATCTTTATTTTT    1020

TATCGTTTATCGTTTAATATAGAATCTCTGTCTTTTCATTCACGTTAGCATAGAGTTTGA    1080
                                                  Bcl I
TCA    1083
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FIG. 2. Nucleotide sequence of 1,083-bp *Nhe*I-*Bcl*I restriction fragment containing the *Suc1*$^+$ gene. The predicted protein sequence of the gene product is shown interrupted by two intervening sequences (dashed lines). Heavy lines under the intervening sequences mark the position of the splice consensus sequences illustrated further in Fig. 5. A filled square and triangle mark the initiation and major termination sites, respectively, of the *Suc1* mRNA transcript.

The 2.7-kb *Hin*dIII restriction fragment was further subcloned as a 1,083-bp *Nhe*I-*Bcl*I fragment which retained *Suc1*$^+$ activity (Fig. 1). The nucleotide sequence of this DNA was determined (Fig. 2; see Materials and Methods). In an attempt to identify the protein-coding sequence of the *Suc1*$^+$ gene, the entire 1,083 bp was scanned by computer for regions of open reading frame (ORF) but was found to contain no ORF longer than approximately 50 amino acids in any of the three frames on either DNA strand. It appears that, if *Suc1*$^+$ encodes a protein product, it is either of very low molecular weight or its mRNA transcript is extensively spliced. The RNA transcript of *Suc1*$^+$ has been identified by Northern blotting (31) and mapped in detail with S1 nuclease (6) to formally predict the primary sequence of the gene product.

A Northern blot revealed that two transcripts of approximately 860 and 950 nucleotides hybridize with a *Suc1* DNA probe (Fig. 3). For S1 mapping of these transcripts, uniformly ^{32}P-labeled DNA probes were prepared by primer extension (10) of single-stranded DNA templates (Fig. 1; see Materials and Methods). Since these probes are uniformly rather than end labeled, they can be used to define breakpoints in an RNA-DNA hybrid only if more than one overlapping DNA probe is used. We therefore prepared a series of seven overlapping single-stranded DNA probes spanning the entire length of the 1,083-bp *Nhe*I-*Bcl*I fragment (Fig. 1; see Materials and Methods).

The 5′ initiation site of the *Suc1*$^+$ transcript was determined with two DNA probes, I and II (Fig. 1). After hybridization with whole-cell RNA and subsequent exposure to S1 nuclease, they yielded protected DNA fragments of 109 and 124 nucleotides, respectively (Fig. 4A). Since the two probes differed in length by 15 nucleotides (*Xba*I site to *Bcl*I site [Fig. 1 and 2]) and the protected fragments of 109

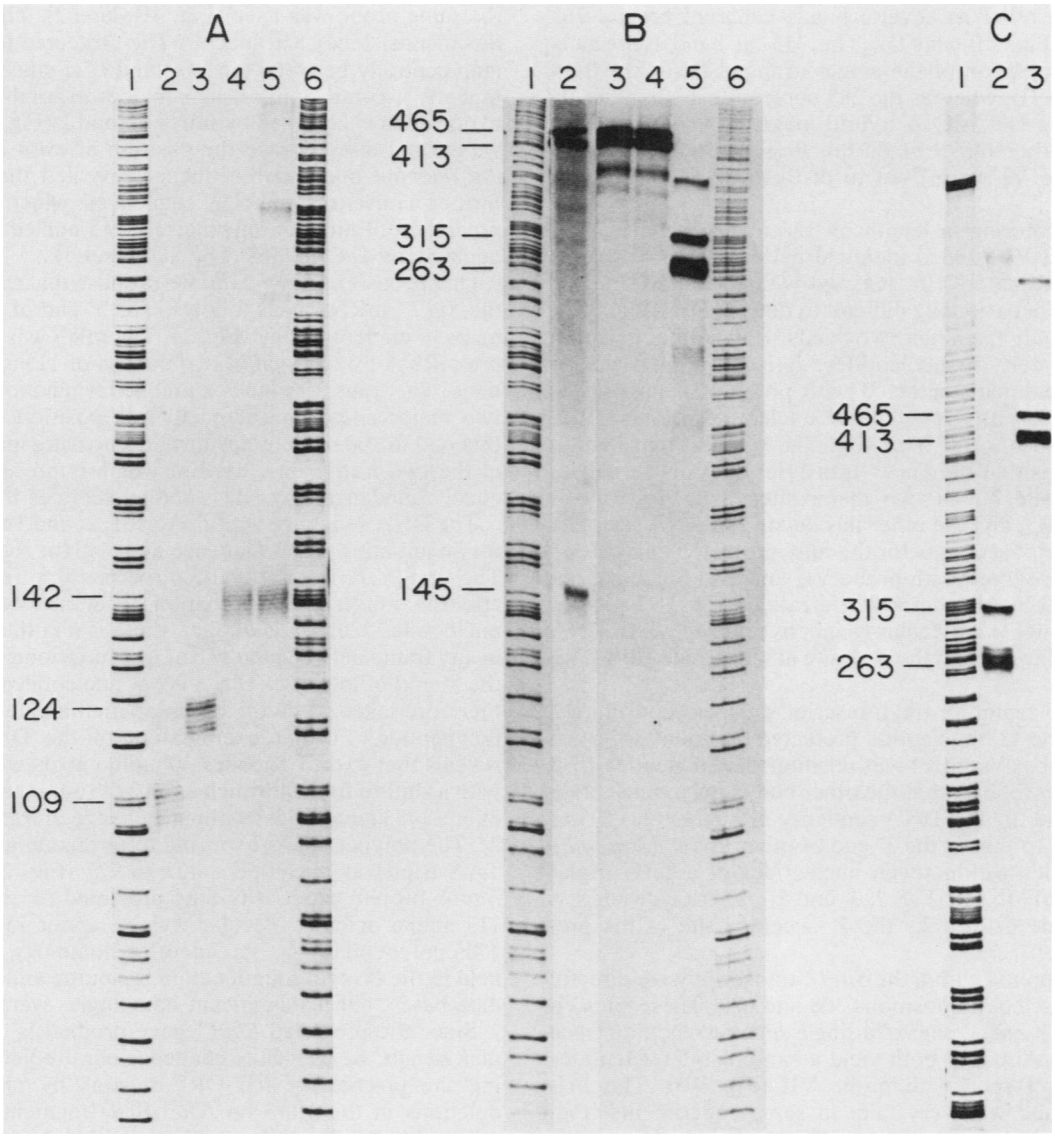

FIG. 4. S1 nuclease protection of *Suc1* transcript. (Panel A). Lanes: 1, A-lane of a sequencing ladder generated from the template used to prepare probe III; 2, probe I protected from S1 nuclease by hybridization to *S. pombe* RNA; 3, probe II; 4, probe III; 5, probe IV; 6, A-lane of sequencing ladder. (Panel B) Lanes: 1, A-lane of sequencing ladder; 2, probe V; 3, probe V (a repeat of the experiment in lane 2; see the text); 4, probe VI; 5, probe VII; 6, A-lane of sequencing ladder. (Panel C) Lanes: 1, A-lane of sequencing ladder; 2, probe VII; 3, probe VI. The data are the same as those in Fig. 4B, lanes 4 and 5, except that the gel contained 4% rather than 6% polyacrylamide and so allowed better resolution of bands in the 200- to 480-nucleotide range. The numbers to the left of each panel indicate molecular size in nucleotides.

and 124 nucleotides differed to precisely the same extent, the RNA-DNA heteroduplex must span both the *Xba*I and *Bcl*I sites (Fig. 1) and dissociate at a position 108 nucleotides from the *Xba*I site, at nucleotide position 167 (Fig. 1 and 2). This region does not contain sequences normally present at sites of RNA splicing (8, 9), and it is most likely that nucleotide 167 instead marks the site of *Suc1*$^+$ transcriptional initiation. The 5′ end of the transcript appears not to be unique but to span a region of seven nucleotides among which position 167 is marginally preferred (Fig. 4A).

Two additional probes, III and IV (Fig. 1), which extended further into the gene than probe II (to the *Eco*RI and *Cla*I sites, respectively), both yielded protected fragments centered around a length of 142 nucleotides (Fig. 4A). This implies that the RNA transcript does not run continuously from the *Bcl*I to the *Eco*RI and *Cla*I sites but breaks at a site 142 nucleotides from its 5′ end at nucleotide position 308 (Fig. 1 and 2). The DNA sequence of nucleotides 309 to 314 closely matches that of other known sites of *S. pombe* pre-mRNA splicing (Fig. 5). Nucleotide position 308 is therefore taken to be the last nucleotide of exon I of the *Suc1*$^+$ gene (Fig. 1 and 2).

The 3′ end of the *Suc1*$^+$ transcript was next considered. This was mapped with three single-stranded DNA probes, V, VI, and VII, each of which extended from the *Bcl*I site at nucleotide 1,083 to either the central *Bcl*I site (V), the *Cla*I site (VI), or the *Ssp*I site (VII). Probe VII yielded two protected fragments, one as a precise band of 315 nucleo-

tides and the other as several bands centered around 265 nucleotides (Fig. 4B and C). The 315-bp band represents full-length protection of the single-stranded *BclI-SspI* fragment (probe VII), whereas the 265-nucleotide band indicates a break in the DNA-RNA hybrid located some 50 nucleotides from either the *SspI* or the *BclI* site. Hybridization probes V and VI were used to distinguish between these alternatives.

Although differing in length by 126 nucleotides (Fig. 1), probes V and VI yielded identical patterns of three bands each of approximately 416, 465, and 145 nucleotides, the last of which was occasionally difficult to detect (Fig. 4B and C). Considering only the larger two bands, which differ in length by approximately 50 nucleotides, it is apparent that the transcript breakpoint detected with probe VII must lie 50 nucleotides from the *BclI* site at nucleotide position 1033 rather than close to the *SspI* site. The simplest explanation of these data is that the *Suc1*$^+$ transcript does not terminate at a unique site but at two major sites. One lies around nucleotide 1033, and the other lies beyond the *BclI* site. The longer transcript accounts for the fully protected band of 315 nucleotides observed with probe VII and also for the upper band detected by Northern hybridization (Fig. 3). Only the transcript of higher molecular weight hybridized with a DNA probe which lay beyond the *BclI* site at nucleotide 1083 (Fig. 3).

The central region of the transcript was next considered. One end of the 413-nucleotide protected fragment observed with both probes V and VI was positioned at nucleotide 1033 (see above). It follows that the other end maps to nucleotide 620 (Fig. 1 and 2). The DNA sequence of nucleotides 603 to 609 is similar to that at the 3′ end of other known *S. pombe* introns and lies within seven nucleotides of a TAG triplet (nucleotides 617 to 619 [Fig. 2, 3, and 5]). These data suggest that nucleotide 620 marks the 3′ acceptor site of the pre-mRNA splice.

Two breakpoints within the *Suc1*$^+$ transcript were directly mapped to nucleotide positions 308 and 620. These sites do not mark the 5′ and 3′ ends of a single splicing reaction, since DNA probes V and VI both yield a band of 145 nucleotides which was not seen with probe VII (Fig. 4B). The 145-nucleotide band was very faint in some experiments (Fig. 4B, lanes 3 and 4) but was unmistakable in others in which the same probe was used (Fig. 4B, lane 2). The reasons for this inconsistency are unclear. The protected fragment must map centrally between the *SspI* and *ClaI* sites. We suppose that the 145-nucleotide fragment corresponds to a central exon which is bounded by introns 1 and 2 (Fig. 1). Although we did not directly map the position of exon 2, a search of the relevant nucleotide sequence revealed the presence of further consensus splicing sequences which allowed the prediction of an exon of precisely 145 nucleotides between nucleotides 424 and 568 (Fig. 1, 2, and 5).

Suc1	**1**	**G/GTATGA**	**TGCTAAT**	**8bp**	**TAG/**	**115bp**
Suc1	**2**	**A/GTAAGT**	**AACTGAC**	**7bp**	**TAG/**	**51bp**
cdc2	1	G/GTAGGT	TACTGAC	6bp	TAG/	68bp
	2	G/GTAAGT	TTCTAAC	8bp	AAG/	76bp
	3	G/GTATAT	CGCTAAC	4bp	TAG/	103bp
	4	T/GTAAGA	TACTAAC	9bp	AAG/	54bp
nda2		G/GTATGT	AGCTAAC	5bp	TAG/	91bp
nda3	1	T/GTACGA	AGCTAAT	4bp	TAG/	36bp
	2	G/GTAAGC	AACTAAC	6bp	AAG/	52bp
	3	T/GTAGGT	TACTGAC	7bp	TAG/	41bp
	4	G/GTAGGT	TACTGAC	5bp	TAG/	89bp
	5	G/GTATGT	TGCTAAC	7bp	TAG/	85bp
ras		G/GTAAGT	AACTAAT	15bp	TAG/	67bp

FIG. 5. *Suc1*$^+$ splicing consensus sequences. Sequences surrounding the 5′ and 3′ ends of introns 1 and 2 of *Suc1*$^+$. The oblique lines mark the inner and outer boundaries of the two introns. The total size of each intron is given. Sequences surrounding the introns of the *cdc2* (15), *nda2* (32), *nda3* (16), and *ras* (12) genes of *S. pombe* are also illustrated.

On the basis of these data, we propose that the structure of the *Suc1*$^+$ mRNA is as follows. The 5′ end of the transcript maps to nucleotide 167 (Fig. 1). The mRNA is derived from pre-mRNA by removal of two introns of 115 and 51 nucleotides. The transcript lacks a unique termination site but has two major sites, one at nucleotide position 1033 and the other 50 to 100 nucleotides further downstream. The shorter of the two transcripts, each of which is present in roughly equal abundance (Fig. 3), is 866 nucleotides long.

The DNA sequence within exons 1, 2, and 3 was examined for an initiating AUG sequence and also for regions of ORF. The first ATG in any of the three possible reading frames after the transcriptional initiation site at nucleotide 167 lies at nucleotides 220 to 222. In fact, there is no other ATG triplet in any frame between the site of transcriptional initiation and the 5′ end of intron 1. The ATG at nucleotides 220 to 222 is therefore taken to be the initiating methionine of the *Suc1*$^+$ polypeptide. Further examination of the DNA sequence reveals that exon 1 encodes 30 amino acids which continue with a shift of frame through exon 2 (48 amino acids) and into exon 3 (48 amino acids) without a change of frame (Fig. 1 and 2). The polypeptide is expected to terminate at the in-frame TAA triplet at nucleotides 725 to 727 (Fig. 2). The entire *Suc1*$^+$ protein product is thus predicted to consist of only 113 amino acids (molecular weight, about 13 kilodaltons). This polypeptide was screened for homology with proteins held in the Protein Identification Resource and the GenBank data bases, but no significant homologies were found.

Since the predicted *Suc1*$^+$ gene product is of low molecular weight, we wished to challenge our predictions concerning the position of the ORF regions by making further deletions in the 1,083-bp *NheI-BclI* fragment and also by constructing frameshift mutations within it. A 674-bp *ScaI-SspI* restriction fragment contains exons 1, 2, and 3 but lacks much of the 3′ untranslated region of the gene. This fragment was inserted into the unique *SmaI* site of pIRT2 and was found to retain *Suc1*$^+$ activity. However, a 323-bp *ScaI-ClaI* fragment carried in the same vector was inactive. This restriction fragment has exon 1 but lacks exons 2 and 3 (Fig. 1).

Two frameshift mutations were constructed within the *Suc1*$^+$ *ScaI-SspI* restriction fragment. The *XbaI* and *ClaI* sites (Fig. 1) were separately cleaved by their respective endonucleases, blunt ended with the Klenow fragment of DNA polymerase, and resealed by ligation. This procedure created a 3 + 1 frameshift at the *XbaI* site (5′TCT AGA→TCTAGCTAGA) and a +2 frameshift at the *ClaI* site (5′ATCGAT→ATCGCGAT). The *XbaI* mutation, which lies within exon 1, lacked *Suc1*$^+$ activity, but the *ClaI* mutation, which is expected to lie within intron 1, was fully active (Fig. 1 and 2). These data are consistent with the presented interpretation of the *Suc1*$^+$ gene and the splicing pattern of its pre-mRNA.

Chromosomal gene disruption and overexpression of *Suc1*$^+$. The only phenotype previously associated with *Suc1*$^+$ is the ability of the plasmid-borne gene to suppress certain temper-

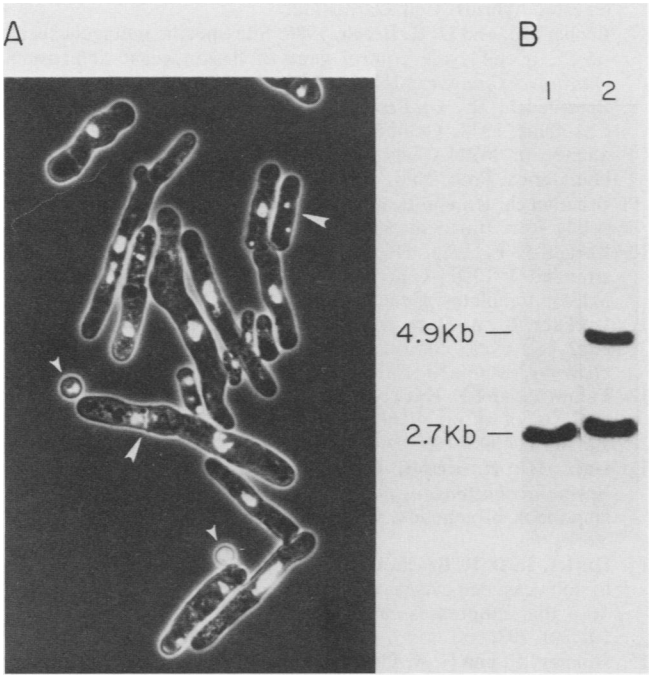

FIG. 6. Chromosomal gene disruption of *Suc1*. (Panel A) Spores derived from a *Suc1*$^+$/*Suc1*::*leu* diploid strain (see the text) germinating in minimal selective medium lacking leucine. The small, round cells (small arrowheads) are presumed to be *Suc1*$^+$ *leu1*$^-$ segregants which fail to undergo cell outgrowth in the absence of leucine. The spores which germinated and grew (large arrowheads) are presumed to be *Suc1*::*leu* segregants. They undergo limited cell division. The cells were fixed in ethanol, exposed to RNase, stained with propidium iodine, and photographed under combined phase-contrast and fluorescence microscopy. The cell nuclei are highlighted by this procedure. (Panel B) Southern blot of DNA from the parental *Suc1*$^+$/*Suc1*$^+$ strain (lane 1) and the *Suc1*$^+$/*Suc1*::*leu* (lane 2) transformant. The DNA of each strain was digested with *Hin*dIII and probed with the 2.7-kb *Hin*dIII fragment containing *Suc1*. The *Suc1*/*1m*$^+$/*Suc1*::*leu* heterozygote contains the expected bands of 2.7 and 4.9 kb, indicating replacement of *Suc1*$^+$ by *Suc1*::*leu* in one of the two chromosomes.

ature-sensitive alleles of *cdc2*. We investigated the phenotype of a null allele of *Suc1* constructed by the method of chromosomal gene disruption in a diploid *S. pombe* strain (28).

The *LEU2* gene of *S. pombe* was inserted into the frameshift-sensitive *Xba*I site within exon 1 of *Suc1*. The construction, referred to as *Suc1*::*leu* (see Materials and Methods) was linearized by restriction endonuclease digestion and introduced by transformation into a diploid *S. cerevisiae* strain (*h*$^{+N}$ *ade6-216 leu1-32*/*h*$^{-S}$ *ade6-210 leu1-32*). Forty-eight Leu$^+$ transformants were picked, and among these one was found to have a stable Leu$^+$ phenotype. DNA was prepared from this transformant and, by the method of Southern blotting (28), the wild-type *Suc1*$^+$ allele was shown to have been replaced by *Suc1*::*leu* in one chromosome of the diploid strain (Fig. 6B).

The *Suc1*$^+$/*Suc1*::*leu* heterozygous diploid was allowed to undergo sporulation, and the resulting meiotic products were examined by free-spore analysis (see Materials and Methods). Spores germinated on minimal selective medium lacking leucine failed to form visible colonies, whereas colony formation was observed on medium supplemented with leucine. However, microscopic examination of the plates revealed that approximately 50% of the spores on leucine-supplemented medium had not formed colonies. Instead, they germinated and either did not divide or at most underwent three rounds of cell division. These microcolonies developed from approximately half of the spores germinating on medium either containing or lacking leucine. Since spores derived from the parental (*h*$^{+N}$ *ade6-216 leu1-32*/*h*$^{-S}$ *ade6-210 leu1-32*) diploid strain underwent no cell outgrowth or division during germination on leucine-free medium but formed colonies with greater than 95% efficiency on leucine-containing medium, we conclude that the microcolony-forming spores derived from the *Suc1*$^+$/*Suc1*::*leu* heterozygote must carry the *Suc1*::*leu* allele.

To examine the terminal phenotype of germinating *Suc1*::*leu* spores in greater detail, a preparation of pure spores devoid of asci and unsporulated cells was obtained from the heterozygous diploid strain carrying *Suc1*::*leu* (see Materials and Methods). The spores were inoculated in liquid minimal medium lacking leucine. As expected, many spores (*Suc1*$^+$ *leu1*) failed to grow but others (*Suc1*$^-$ *leu*$^+$) underwent limited cell division. Microscopic examination of the culture 24 h after inoculation revealed that many but not all of the germinated cells had elongated to several times the normal length at cell division (Fig. 6). This phenotype is characteristic of *S. pombe* cells, which are capable of growth but are arrested in progression through the cell division cycle.

Suc1$^+$ was initially identified as a plasmid-borne gene that could suppress certain alleles of *cdc2* (14) but otherwise had no other overt phenotype. To test the effects of more extreme overexpression of *Suc1*$^+$, the gene was placed adjacent to the promoter of the *S. pombe* alcohol dehydrogenase gene (adh), and the construction was introduced into *S. pombe* by chromosomal integration (see Materials and Methods). Such transformants had a clear phenotype. Cell growth was minimally affected, but division occurred at an average length of 30 μm, approximately twice the normal cell length. Extreme overexpression of *Suc1*$^+$ thus caused a delay in completion in the cell division cycle.

DISCUSSION

The *Suc1*$^+$ gene is slightly unusual in that it appears to encode a protein product which, at 13 kilodaltons, is smaller than any previously described gene product in *S. pombe*. A prediction of a low-molecular-weight protein would obviously tend to arise if errors, particularly frameshift errors, were introduced during DNA sequencing. However, we believe that the presented sequence of 1,083 bp is fully correct. Once no extended ORF regions had been detected, much of the sequence was redetermined at Cold Spring Harbor Laboratory and no discrepancies were found.

S1 mapping revealed that the 866-nucleotide *Suc1*$^+$ transcript is interrupted by two introns. The predicted positions of the boundaries of exon 2 are based in part on knowledge of consensus sequences surrounding pre-mRNA splice sites. Such consensus sequences are not absolutely conserved and tend to be more variable in *S. pombe* than in *S. cerevisiae* (12, 15–17, 32). However, within the relevant region of sequence, no other placement of a 145-nucleotide exon would allow observance of even the limited 5′ GT and 3′ AG rule which appears to be followed in all eucaryotic introns (9).

The predicted 113-amino-acid product of the *Suc1*$^+$ gene was not found to share significant homology with protein

sequences held in widely available data bases, and therefore no indication of its possible biochemical role was provided. However, we established that the *Suc1*$^+$ product is essential for cell viability. Spores carrying a null allele of *Suc1* (*Suc1*::*leu*) were capable of very limited cell division. The few divisions which occurred may merely reflect the gradual exhaustion of a limited amount of *Suc1*$^+$ gene product which presumably partitions into *Suc1*::*leu* spores during their formation. The terminal phenotype of cells carrying *Suc1*::*leu* was not unequivocal. Complete inhibition of cell growth during spore germination results in very small, round cells. For example, *leu1-32* spores germinating in the absence of leucine displayed exactly this phenotype (Fig. 6). On the other hand, complete inhibition of the cell division cycle in the absence of any growth defect results in uniformly elongated cells. Such is the terminal phenotype of spores carrying a null allele of *cdc2* (7). The terminal phenotype of *Suc1*::*leu* spores appears to be a combination of growth and cell division cycle defects. Once the germinating spores had ceased cell division, some cells became highly elongated, whereas others did not (Fig. 6). No attempt was made to determine the stage in the cell cycle at which *Suc1*::*leu* spores became arrested because of the heterogeneity in their terminal phenotype. Paradoxically, strong overexpression of *Suc1*$^+$ also resulted in cell elongation. However, overexpression of *Suc1*$^+$ was not lethal, and the observed elongation of cells indicated that completion of the cell cycle was delayed but not fully inhibited.

The experiments described above gave no indication of the biochemical role of the *Suc1*$^+$ gene product and, in particular, its relationship to the *cdc2*$^+$ gene product. However, nucleotide sequencing of the *Suc1*$^+$ gene and prediction of the primary sequence of its product allows anti-*Suc1* antibodies to be raised. Such antibodies will allow possible interactions between the *Suc1*$^+$ and *cdc2*$^+$ gene products to be investigated. Furthermore, it will also be possible to use the isolated *Suc1*$^+$ gene to construct conditional lethal and other alleles of the gene to better characterize its role in cell growth and division.

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